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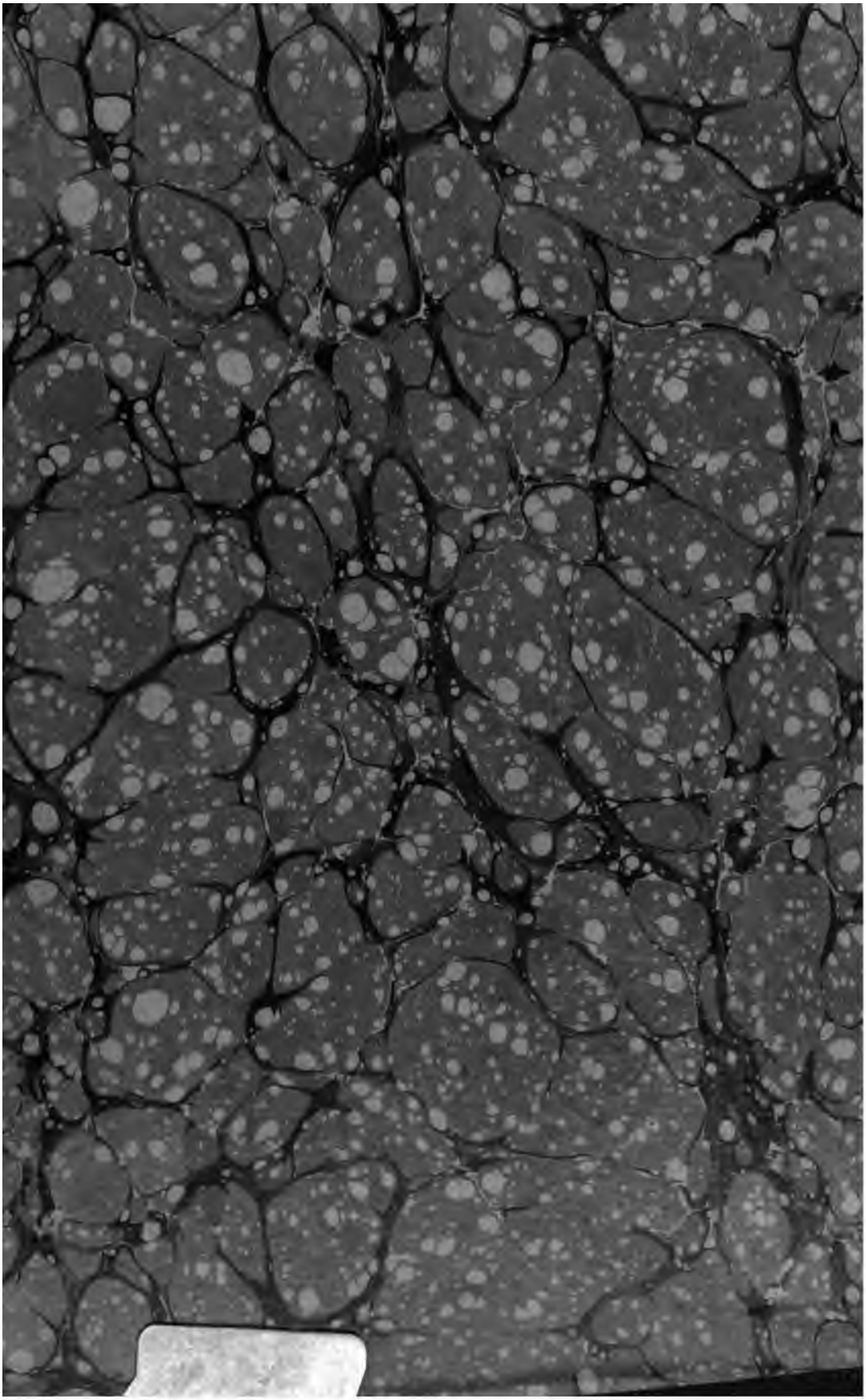

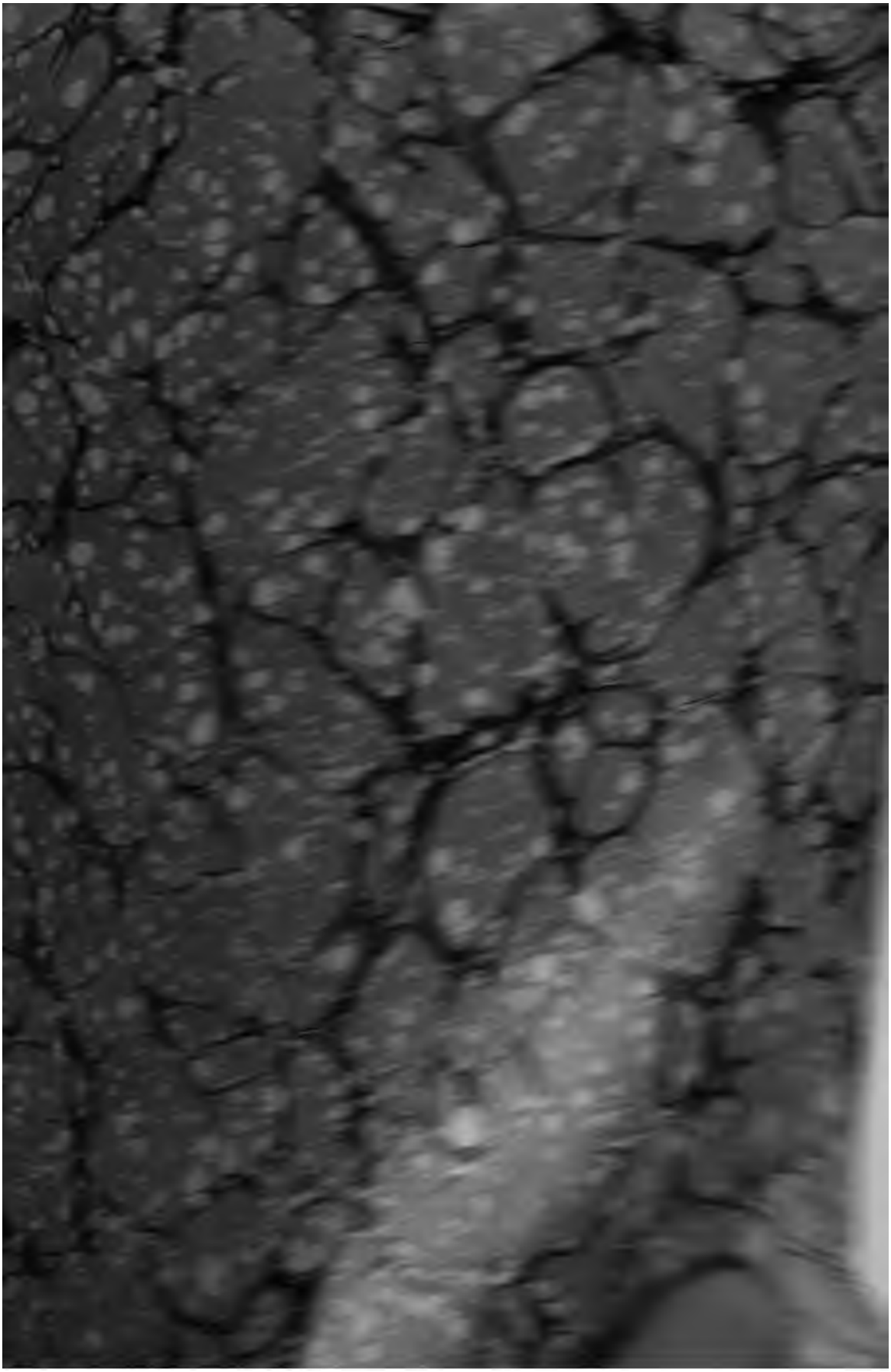

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OF THE

Marine Biological Laboratory

WOODS HOLL, MASS.

VOL. XI JUNE, 1906 NO. 1

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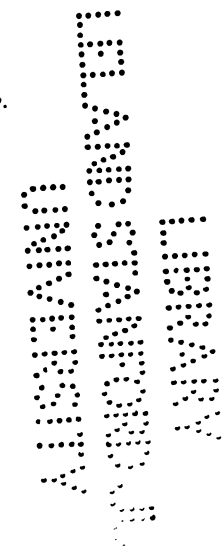

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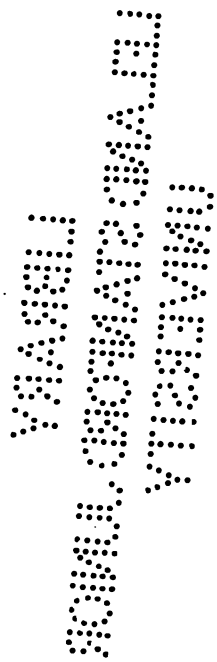

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BIOLOGICAL BULLETIN

THE NASO-LABIAL GROOVE OF LUNGLESS SALAMANDERS.

INEZ L. WHIPPLE.

So far as I have been able to ascertain, there is no mention in scientific literature of a very definite external feature of certain salamanders to which, because of its location, I have given the name, *naso-labial groove*. This groove, which extends from the latero-ventral angle of the external naris in a practically vertical direction to the edge of the upper lip, I have found in the following species, a list which includes nearly every genus of the *Desmognathidæ* and *Plethodontidæ*, all, in fact, which I had the opportunity to examine:

PLETHODONTIDÆ.

Batrachoseps attenuatus,
Plethodon erythronotus,
Gyrinophilus porphyriticus,
Spelerpes bilineatus,
Spelerpes guttolineatus,
Autodax lugubris,
Plethodon cinereus,
Plethodon glutinosus,
Manculus quadridigitatus,
Spelerpes longicaudus,
Spelerpes ruber,

DESMOGNATHIDÆ.

Desmognathus fusca,
Desmognathus brimleyorum.

This feature is not only apparently characteristic of the *Plethodontidæ* and *Desmognathidæ*, but is evidently peculiar to these families. None of the representatives of the other families of the *Salamandrida*[1] which I have examined possess it, neither

[1] The species of salamanders in which I have found no naso-labial groove are as follows: *Salamandra maculosa*, *Triton cristatus*, *Triton alpestris*, *Triton helveticus*, *Diemyctylus viridescens*, *Amblystoma punctatum*, *A. opacum*, *A. talpoideum*, *A. jeffersonianum*, *Salamandrina perspicillata*.

have I been able to find in any of the *Derotremes* or the *Perennibranchs* any trace of it.

Moreover, since all of the species which are known to possess the groove are lungless, and, since of those which do not possess it only one, *Salamandrina perspicillata*, lacks functional lungs, it is evident that the groove has some function associated with respiratory habits and correlated with the absence of lungs.

Description of the Naso-Labial Groove and Associated Structures.

In my study of the naso-labial groove and the organs associated with it I have used mainly *Desmognathus fusca*, the species which I have been able to obtain in largest numbers, both in the adult and larval stages. The following description applies, therefore, particularly to this species.

The methods of study employed have included much observation of the living animals under varied conditions, gross dissection, and the microscopic study of both horizontal and transverse series of sections. Horizontal sections are particularly favorable for the study of the groove, since in these it is cut transversely.

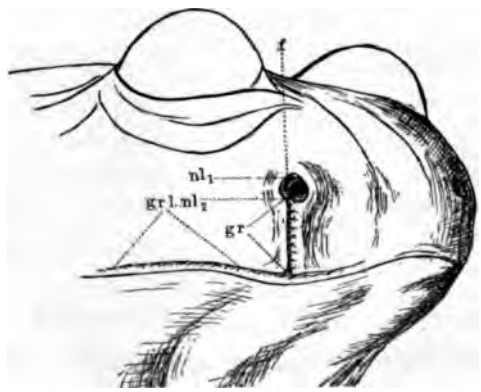

FIG. 1. Head of adult *Desmognathus fusca* showing external naris and naso-labial groove. *f*, crescentic fold; *gr*, naso-labial groove; *grl*, labial (horizontal) groove; nl_1 and nl_2, orifices of first and second naso-labial glands. Other orifices may be seen along the mesial side of the naso-labial groove and along the horizontal groove.

Externally the naso-labial groove (Fig. 1, *gr*), though a mere line, may be easily seen even with the unaided eye, its conspicu-

ousness being usually emphasized by the absence of pigment along its course. It begins at the latero-ventral angle of the external naris of which it is in a sense an exaggerated ventral elongation. From this point it extends ventrally until it reaches the edge of the lip, where it ends in a somewhat vaguely defined horizontal groove (Fig. 1, *grl*), which meets it at right angles. This horizontal groove is parallel with the edge of the lip and gradually disappears as it approaches the mid-line on the one hand and the angle of the mouth on the other.

In its connection with the external naris the naso-labial groove shows a very definite relationship to certain structures which have been worked out and described by Bruner ('96). This investigator has shown that the *Salamandrida* possess in connection with the external naris a set of involuntary muscles for opening and closing the orifice, the *M. constrictor naris* and *M. dilatator naris* respectively. The constrictor is situated in the lateral wall of the nasal cavity with its fibers partly encircling the orifice, so that externally the visible effect of its contraction is to draw a crescentic fold of skin (Fig. 1, *f*) across the orifice from the outer toward the inner border, the naris being thus partially or completely closed according to the amount of contraction. By the contraction of the dilatator muscle, the fold is again pulled back toward the outer boundary of the naris. When the orifice is closed the crescentic fold forms the floor of a slight depression which is completely encircled except at the latero-ventral angle, the point from which the naso-labial groove leads.

Associated with the external naris and with the naso-labial groove are a number of well developed glands which I shall speak of collectively as the *naso-labial glands*. Although these glands show much individual variation, the location of their orifices is practically constant for the species, the first two being specially definite. The more dorsal of these (Fig. 1, nl_1) is in the dorsal angle of the naris, that is, at the end of the crescentic fold. The second one (Fig. 1, nl_2) is on the ventral edge of the nasal orifice at the point where the groove joins the naris and therefore upon the mesial side of the groove. Arranged at approximately regular intervals along the mesial border of the groove are other orifices, from six to twelve in number, and similar orifices occur

along the ventral border of the horizontal groove into which the naso-labial groove leads. The orifices associated with the grooves are usually visible externally through a lens, since each is situated in the middle of a little elevation.

Seen in transverse section, that is, in a series of horizontal sections through the head, the naso-labial groove appears as an infolding of the entire epidermis (Figs. 2, 3 and 4, *gr*), the deeper

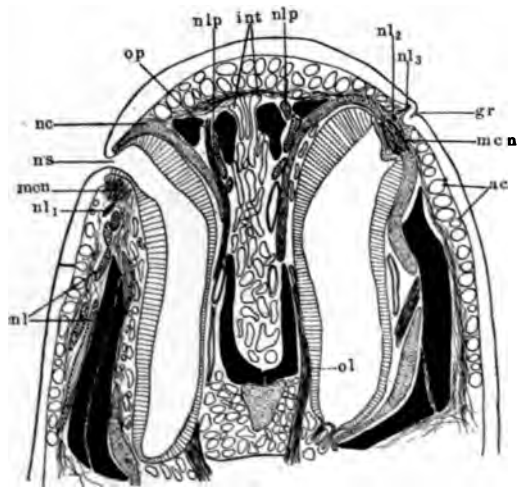

FIG. 2. Horizontal section through head of adult *Desmognathus fusca*, × 20. *ac*, acinous glands of skin; *gr*, cross-section of naso-labial groove; *int*, intermaxillary glands; *mcn*, M. constrictor naris; *nc*, nasal capsule; *nl*, naso-labial glands; nl_1, nl_2, nl_3, the first, second and third naso-labial glands respectively; *nlp*, tubules of naso-labial glands within the premaxillary foramina; *ns*, external naris; *ol*, olfactory nerve; *op*, internal nasal branch of ophthalmic nerve.

portion of which has a curved outline, while the outer portion dips abruptly in, giving the groove in transverse section the appearance of the letter V. It is deeper and narrower near the naris and becomes wider toward the edge of the lip. Underlying the groove is a dense mass of connective tissue (Fig. 3, *c*) richly supplied with blood vessels.

In these horizontal sections the naso-labial glands (*nl*) are seen to make a vertical descent through the epidermis and then to curve sharply and extend in either a general mesial or lateral direction beneath the skin and parallel with it. Although it is

often difficult to distinguish in sections between the narrow ducts of these glands and those of the large acinous glands (*ac*) with

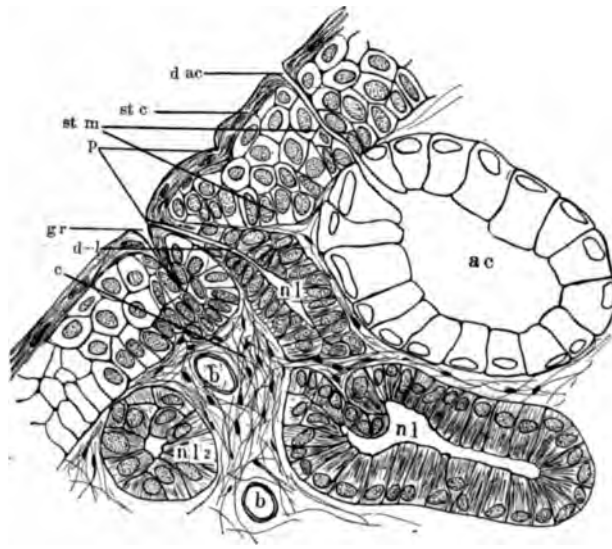

FIG. 3. Transverse section through left naso-labial groove of adult *Desmognathus fusca*, × 237.5. *b*, blood vessels; *c*, connective tissue underlying the naso-labial groove; *dac*, duct of acinous gland; *dnl*, duct of naso-labial gland; *p*, papilla upon the surface of which the naso-labial gland opens; *stc*, stratum corneum; *stm*, stratum mucosum. Other designations as in Fig. 2.

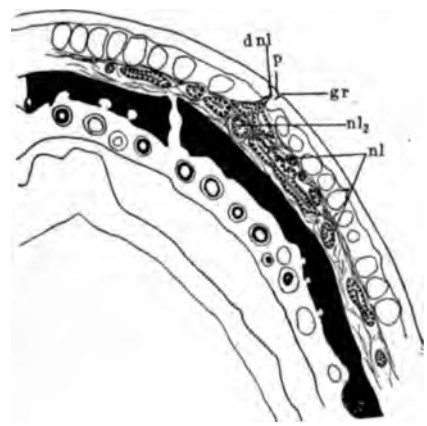

FIG. 4. Horizontal section through right side of head of a small adult, *Desmognathus fusca*, × 42. Designations as in previous figures. The second gland (nl_2) is cut through the portion which runs for a short distance parallel to the groove and just beneath it (cf. Fig. 5).

which the skin is thickly studded, the glandular portions are readily distinguished by the smaller size of the naso-labial glands and of their lumina, as well as by their deeper situation. The acinous glands are a part of the skin and lie within it; the naso-labial lie beneath the skin.

In working out the course of the glands two methods were used. The more reliable one as to exact relationships was the serial-section method by means of which the complete course of each tubule may be worked out. Several series both horizontal and transverse were used in this way. The disadvantage of this method lies in the fact that since the glands are of small diameter, much convoluted, and in some regions closely packed together, it is necessary, in order to obtain reliable results, to have thin sections and an absolutely uninterrupted series, conditions which are not easily fulfilled in sectioning an entire head. Even with a

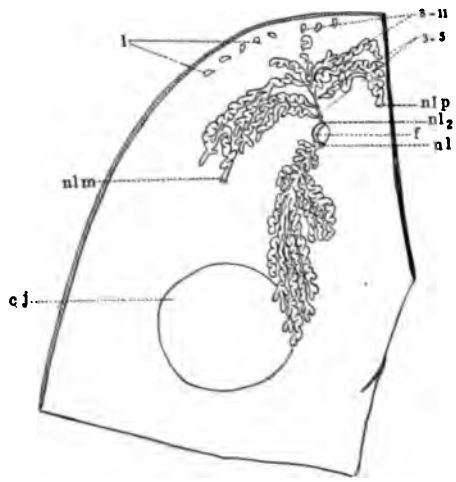

FIG. 5. Dissection of individual shown in Fig. 1, in which the skin is removed from the right side of the head, the naso-labial glands being removed with it. Drawing shows under surface of skin with glands in place. *cj*, conjunctiva; *l*, labial glands; 3–5 and 8–11 indicate the enumeration of the naso-labial glands; *nlm*, cut end of tubule which lies in the groove of the maxillary bone. Other designations as in previous figures.

perfect series, moreover, it is extremely laborious to trace even a single tubule throughout its tortuous course. Gross dissections were, therefore, found to be of great value as a means of corrob-

oration of results obtained from the study of serial sections. It was found possible, with alcoholic specimens, to dissect off the skin from the entire head, removing with it all of the soft parts, eyeballs included, external to the skull; and such dissections, while always involving more or less injury to some of the tubules, were still sufficiently good so that, when the skin was pinned out under water and further dissection and separation of the glands accomplished, practically every anatomical fact which had been learned from the sections could be demonstrated.

The naso-labial glands are tubular glands much convoluted, and showing a tendency to develop acini at the bends of the tubules. The different glands of the group show great inequality both in complexity and in length of the tubules. The longest and most complicated are the two which are associated with the naris itself [referred to as (nl_1) and (nl_2) in the figures]. Both of these are not only much convoluted but are branching. The dorsal gland (nl_1) often sends several branches as far posteriorly as the orbit, over the edge of which a few tubules usually find their way, while some may even extend entirely across the inner surface of the eyeball. The duct of the second gland (nl_2) extends ventrally for a little distance beneath the groove, and then gives off almost at right angles several branches both mesial and lateral. The lateral have been in some specimens traced in a course parallel with the edge of the lip as far posteriorly as the eyeball, while the mesial extend nearly to the mid-line and then may turn in a dorsal direction.

Of the remaining naso-labial glands (Fig. 5, *3–5*, *8–11*), some are several millimeters in length, others much shorter. All, however, are long enough to be classified as tubular glands. The shorter glands are in general those opening along the more ventral portion of the groove, and along the horizontal groove (*grl*) only very short ones have been observed. All of the longer tubules lie beneath the skin parallel with the edge of the lip, extending either mesially or laterally from the groove, and they sometimes equal in length the single branches of the large gland (nl_2) which opens on the ventral border of the naris. Occasionally a mesial and a lateral tubule unite and open by a single duct (Fig. 4, *dnl*), thus forming a branching gland.

In two regions the naso-labial glands come into close proximity with other glands of such similar structure that the determination of the actual relationship is very perplexing. One, at least, of the mesial tubules (Figs. 2 and 6, *nlp*),[1] turns dorsally

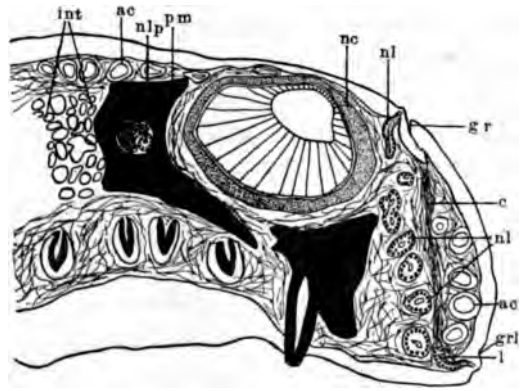

FIG. 6. Transverse section through head of adult *Desmognathus fusca* (× 42), showing the naso-labial groove cut obliquely and the horizontal groove (*grl*) cut transversely. *pm*, premaxillary bone. Other designations as in previous figures.

before reaching the mid-line and enters the foramen for the internal nasal branch of the ophthalmic nerve at the anterior end of the premaxillary bone. Through this foramen the tubule may be traced as far as the opening of the foramen into the intermaxillary space. In this space lie the much convoluted tubules of the intermaxillary gland (*int*), the orifice of which is in the mid-line of the roof of the mouth, a little anterior to the posterior nares. In the larger specimens the naso-labial tubule passes into the intermaxillary space and intermingles its convolutions with those of the intermaxillary gland. I have found it impossible from my preparations to determine whether the tubules of the two glands thus curiously intertwined become actually continuous or not. If such an anastomosis exists, however, it is certainly established secondarily, for in small adults the naso-labial tubule has not reached the intermaxillary space but ends blindly within the foramen, and there is consequently

[1] In every case in which I have been able to demonstrate the relationships of this tubule with absolute certainty, it has proved to be one of the branches of the gland which opens on the ventral margin of the naris (nl_2).

here an unmistakable interval between the two glandular masses.

The other region in which one of the naso-labial glands may become closely related in position to other glands is in the lower part of the orbit where there is a large glandular mass which opens by several orifices on the inner surface of the lower lid. These glands I shall speak of collectively as the *orbital glands*. The tubules of these glands, following the same principles of growth along lines of least resistance as do the naso-labial glands, push their way around both the anterior and the posterior angles of the orbit. The anterior orbital glands and the ultimate branches of the most dorsal naso-labial gland may, therefore, lie side by side within the orbit behind the eyeball. In no case, however, has any trace of coalescence been observed; the tubules of each gland end blindly. Here, again, the condition in the small adults substantiates the conclusion that the association of the glands is merely one of proximity, since in the young adults the naso-labial gland has not reached the orbit and therefore its tubules do not even lie beside those of the orbital gland.

This peculiar relation of the naso-labial to other glands has, therefore, in all probability no significance whatever beyond the fact that it is the tendency of all these glands to work their way into whatever crevice happens to lie in their course. Thus certain of the lateral tubules of the naso-labial glands (Fig. 5, *nlm*) usually find out the foramen in the maxillary bone for the external nasal branch of the ophthalmic nerve and enter it; and the same tendency is shown also by the intermaxillary gland, some of the tubules of which escape from the intermaxillary space through the anterior fontanelle and extend anteriorly immediately beneath the skin of the snout, while other tubules of the same gland escape through the nerve foramen which leads back into the posterior part of the nasal capsule, and thus creep into the cavity under the nasal epithelium in which the internal nasal glands are located.

So far as can be seen externally the naso-labial groove in other species does not differ essentially from that of *Desmognathus fusca*. The relation to the external naris is always the same, but the method of termination at the edge of the lip varies

somewhat. Thus in *Plethedon* the groove gradually becomes less distinct and finally flattens out and is lost upon the surface of a slight labial eminence.

I have worked out the glands by dissection in several species. They all show the same general character, and the position of the orifices of two especially large glands with relation to the naris is practically constant and similar to that in *Desmognathus* (nl_1 and nl_2 of the figures).

The location of the remainder of the orifices is difficult to determine. The position along the mesial border of the naso-labial groove, while a common one, does not seem to hold exclusive possession in all species. The glands do, however, open always in close proximity to the groove, and so far as I have been able to determine, never within it. From the groove the glands have the same mesial and lateral course, parallel with the lip. Some of the glands are always extensive, usually reaching nearly or quite as far posteriorly as the eye. In *Plethodon* several of the

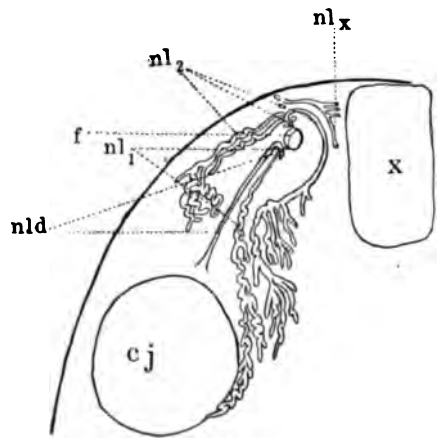

FIG. 7. Dissection showing the naso-labial glands in place on under surface of skin removed from the right side of the head of an adult *Plethodon*. X, the area occupied by a mass of tubules partly from the intermaxillary gland and partly from the naso-labial (nl_x). Other designations as in previous figures.

mesial tubules (Fig. 7, *nlx*) turn dorsally before reaching the midline and extend posteriorly over the surface of the snout, intermingle with the intermaxillary, the tubules of which extend

out through the anterior fontanelle, and finally send several tubules laterally as far back as the dorsal edge of the orbit.

The naso-labial glands are in no sense a new discovery. Under various names they have been described at least in part by Wiedersheim ('76), Seydel ('95), Born ('77) and Bruner ('96 and '01). Wiedersheim ('76) in particular mentions the enormous extent of large external glandular masses in various species of *Plethodon*, *Spelerpes*, *Batrachoseps*, *Gymnophilus* (*Gyrinophilus ?*) and *Chioglossa*. It is interesting to note with regard to this list, made eighteen years before the discovery that any of the salamanders are lungless, that the first four of the genera are now known to be lungless while so far as I know the fifth, *Chioglossa*, has not been investigated as to a possible lungless condition.

Other investigators, particularly Bruner ('96) mention under the discussion of *external nasal glands* in general, some of the *Plethodontidæ* and *Desmognathidæ* as genera in which such glands occur. The glands to which Bruner referred were undoubtedly the two of the naso-labial group which open in connection with the naris. I cannot find in these authors, however, any indication of an appreciation of the great difference in size and importance between these glands of lungless forms and those similarly named which occur in the lunged forms. Bruner, in fact, in discussing the function of the external nasal glands mentions particularly that the extent of the glands exceeds very little that of the *apertura nasalis externis*, a fact which is in general true of the lunged salamanders but is, as has been shown above, far from true in the case of the members of the lungless families. Moreover, the course of the glands in the lunged species which I have examined lies between the nasal capsule and the maxillary bones, while in the *Desmognathidæ* and *Plethodontidæ* these glands are in the main external to all the skeletal parts.

With regard also to the exact location of the orifices of these naso-labial glands the facts seem not to have been clearly and completely stated. Wiedersheim ('76), indeed, located the orifices of the glands in *Batrachoseps* and *Spelerpes* upon the border of the upper lip. He was probably misled, however, by the close association of the ultimate gland tubules with those of the

orbital glands, into the opinion that in *Batrachoseps* at least, the glandular mass had orifices upon the surface of the conjunctiva also. I have not myself sectioned *Batrachoseps* but from the relationships which I have worked out from sections of both *Desmognathus* and *Plethodon* I am convinced that the orbital glands are absolutely distinct and separate from the naso-labial in these forms.

Bruner's ('96, *b*) observations as to the orifices of the glands were correct so far as they went in that these were located in common with those of the external nasal glands of other salamanders, on the border of the naris. His observations, however, did not extend to those of the glands which open along the naso-labial groove.

Development of the Naso-Labial Groove and Glands.

The naso-labial apparatus is essentially an adult characteristic. In large *Desmognathus* larvæ (25 mm.) collected in early summer and only a few millimeters shorter than the smallest adults which I have found (33 mm.) the groove has hardly begun to develop and the anlagen of only the largest of the glands are to be found. During the two months which follow, these structures undergo rapid development, so that the little adults which abound

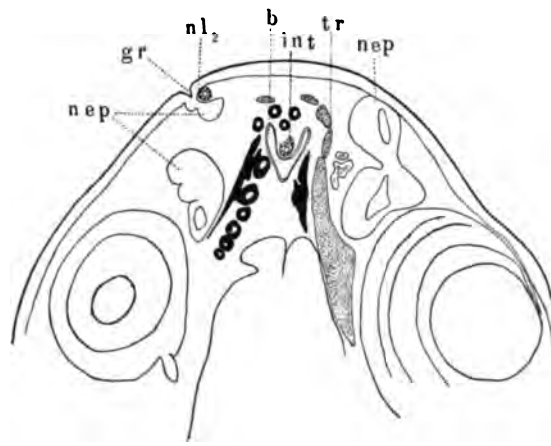

FIG. 8. Horizontal section through the head of a *Desmognathus fusca* larva, cut slightly obliquely. The left side of the section passes just below, the right slightly above, the external naris, × 42. *nep*, nasal epithelium; *tr*, trabecula. Other designations as in previous figures.

in early autumn along the banks of the brooks in which the larvæ live, have developed, with the assumption of terrestrial habits, these distinctively adult characteristics, namely, naso-labial grooves and well developed glands opening in connection with them.

There exist, however, even in the larvæ, certain indications that the process of formation of a naso-labial groove has begun. There is a ventral elongation of the external naris, which really forms a very short groove (Fig. 8, *gr.*). Ventral to this there may be seen by following the horizontal sections for a short distance a slight incurving of the epidermis with a tendency here and there toward the proliferation of cells (*pr*) of the lower of the two layers, a suggestion, at least, of the beginnings of future

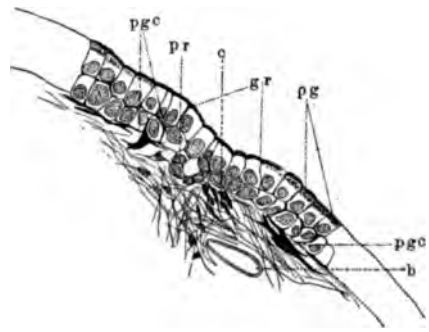

FIG. 9. Detail of a horizontal section through the head of a *Desmognathus fusca* larva ventral to the external naris, showing an early stage in the formation of the naso-labial groove, × 237.5. *pg*, pigment within the cells of the epidermis; *pgc*, pigment cells below and between the cells of the epidermis. Both forms of pigment are absent from the region in which the groove is developing (*gr*) *pr*, proliferation of cells from the under side of the epidermis, probably the anlage of a gland. Other designations as in previous figures.

glands. Noticeable is the absence of pigment in this region both in the case of the epidermal cells and the connective tissue beneath the epidermis. The first glands to appear, and at this stage the *only* ones present, are the two connected with the naris. These are very short, the dorsal extends posteriorly through three or four 10μ sections, the ventral one extends mesially and is very probably the branch which later finds its way into the intermaxillary space.

The small adults, 33 to 45 mm., have the groove well developed. The number of glands connected with it seems to increase

gradually as the animals grow older. The smallest showed three or four only, the more ventral of these being very short. A specimen 43.5 mm. long had six in connection with each groove, while in a large adult as many as twelve are often present.

The extent of the glands also increases as the animals grow larger, a fact which has already been mentioned as throwing light upon the relation of the glands to other glandular masses. One very interesting fact is that the tubule which enters the nerve foramen in the premaxillary does this previous to the roofing over of the foramen. It thus lies at first on the surface of the premaxillary in a mere groove which later becomes enclosed to form a foramen.

The Homology of the Naso-Labial Glands.

There seems to be no reason why the homology which Bruner ('96) has assumed to exist between the external nasal glands of all of the *Salamandrida* and *Anura* should not be accepted for the *Desmognathidæ* and *Plethodontidæ*, in spite of the greater extent and more external position of the glands in these families. This homology will of course include strictly only those two of naso-labial group which open upon the border of the naris, and is based upon similarity of origin and structure between these and the external nasal glands of lunged forms. There is, however, absolutely no reason for considering the remainder of the naso-labial glands as in any essential unlike these two, and the entire group must, of course, be homologized together.

Moreover, I have obtained sufficient evidence (which will be given in a separate paper) to prove that the large gland which occurs in *Amphiuma* and was described by H. H. Wilder ('91) under the name of the "*glandula lateralis*," is also the homologue of these glands as well as of *Oppel's* glands of *Proteus*. The homology of all these glands becomes especially emphasized when we recall that in many of the lunged forms the external nasal glands consist of many tubules opening by separate orifices. Thus Bruner reports for one specimen of *Amblystoma* fifteen independent tubules.

To go back of the adult condition and determine the source from which these external nasal and naso-labial glands were

originally derived, and to decide the question as to their possible relation in origin to other glandular structures such as the acinous glands must, however, be left to further investigation.

The Function of the Naso-Labial Groove and Its Associated Glands.

As expressed in the introductory paragraph, the exclusive occurrence of so definite and well developed a set of organs as the naso-labial groove and glands in those families which are characterized by absence of lungs must be correlated with the lungless condition. The key to the explanation of their functions will be found, therefore, in a study of the respiratory habits, particularly those which involve a use of the nasal passages.

Although certain species of the *Plethodontidæ* and *Desmognathidæ* have an aquatic larval life, practically all are terrestrial in the adult stage. They live in moist places under stones and decaying wood or, like *Desmognathus*, burrow in loose, moist earth along the banks of small streams. Some species, among them *Desmognathus fusca*, do not even go into the water to deposit their eggs. *Autodax lugubris* has carried its terrestrial habits to such an extreme that it has become arboreal, and rears its young in the cavities of tree trunks [Ritter and Miller ('99) and Ritter ('03)]. I have been able to find in the stomachs of both *Desmognathus fusca* and *Plethodon cinereus* only spiders, mites and terrestrial insects, and I am led to infer that the food of these species consists wholly of terrestrial forms.

Whatever other methods of respiration the lungless salamanders may have, they maintain, so long as they have access to air, a constant bucco-pharyngeal aërial respiration. This is approximately regular and is very rapid, sometimes exceeding a hundred respirations a minute, although the number may be as low as fifty. It is accomplished by an alternate depression and elevation of the floor of the tightly closed mouth, as a result of which air is drawn in and expelled through the nasal passages. Slight occasional fluctuations of the crescentic fold which guards the external naris may be observed, but, under ordinary conditions, not a complete closure. If, however, the specimen be immersed in water, or if a drop of water be applied to the snout so

as to flow over the external nares, these immediately close, while bucco-pharyngeal respiration at once ceases and the floor of the mouth is forcibly indrawn, causing, often, the expulsion of tiny bubbles of air through the nares which open sufficiently to permit their escape. All entrance of water into the nasal passages is, by this prompt closing of the nares, effectually prevented. There is, moreover, an independent control of the two nares, since, when the animal is so manipulated that only one naris is covered with water, this one alone is closed, while, through the other, aërial respiration continues. Especially noticeable is the almost immediate resumption of aërial respiration, when, after the interruption caused by immersion, the snout is thrust out of water sufficiently to bring the nares above its surface. Even if only a single naris gains access to the air, this alone opens and the bucco-pharyngeal movements begin again.

All of the forms which I have experimented with, however (*i. e.*, various species of *Plethodon*, *Desmognathus* and *Spelerpes*), show, with the exception of *Spelerpes ruber*, a strong aversion to deep water. When placed in water of too great depth to allow them to raise the tip of the snout above the surface while they are standing upon the bottom, they invariably make frantic efforts to escape. They swim rapidly to the top and hold themselves there by active swimming motions. When unable to sustain themselves longer at the surface, since, in the absence of lungs, they have no hydrostatic powers, they fall to the bottom where they remain for a longer or shorter period of rest. During all these manœuvres the tightly closed condition of the nares and the elevated position of the floor of the mouth are maintained. Moreover, from my observation of *Desmognathus fusca* and *Spelerpes bilineatus* in their natural environment, I am convinced that these species, at least, never voluntarily seek deep water; and when driven into deeper parts of the stream, they soon make their way under cover of fallen leaves and débris to a region where the depth is not too great to allow the snout to be thrust out of water. Under this condition they are perfectly at ease and will remain with the body in water for a long time if only the nares have access to the air so that bucco-pharyngeal respiration may continue.

It is, however, an interesting fact that although bucco-pharyngeal aërial respiration may be necessary to the comfort of these lungless forms, it is apparently not an absolute physiological necessity. I made the experiment of confining individuals representing several species of *Desmognathus*, *Plethodon* and *Spelerpes* in wire cages which were kept immersed in running water with no possible access to air for periods ranging from one to two weeks in length. Although when liberated at the end of the experiment they invariably swam immediately to the surface and made the same vigorous efforts to escape that they had made previous to the experiment, they showed no signs of impairment of physical function and continued to live afterwards in the terrarium with no evidence of ill effects from the ordeal. They were never discovered, however, to open the nares during the confinement under water, or to show any other than the tightly indrawn position of the floor of the mouth.

Spelerpes ruber, unlike most other lungless forms, shows a a decided adaptability to aquatic life, and will live for weeks at the bottom of an aquarium. In this case, however, as in those of less aquatic forms, under compulsory immersion, it is a significant fact that *not only were the external nares apparently kept tightly closed so long as the animal was under the water*, but when finally removed from the water and given access to the air *the resumption of aërial bucco-pharyngeal respiration was immediate.*

The importance of these points for the present discussion will be emphasized by comparison with the habits and behavior of lunged salamanders under similar conditions. All of the lunged salamanders have, in addition to pulmonary respiration, a bucco-pharyngeal aërial respiration exactly similar to that of the lungless forms. When these lunged salamanders are placed under water, however, although there is at first an attempt to exclude the water from the nasal passages by closure of the external nares, these are sooner or later opened and aquatic bucco-pharyngeal respiration is established similar mechanically to the aërial method, though much slower. Usually there is the additional difference that the water is expelled in part through the slightly opened mouth, and in some few instances it has been observed to enter, also, through this channel as well as through the nares.

The efficiency and readiness of this aquatic bucco-pharyngeal respiration varies much with the aquatic habits of the individual species, *Diemyctylus viridescens*, which is thoroughly aquatic, showing one extreme, *Amblystoma opacum*[1] the other.

After the establishment of aquatic bucco-pharyngeal respiration in these forms, the removal from water to air is not accompanied, as in the case of the lungless salamanders, by an immediate resumption of aërial respiration. On the contrary there is often much delay arising from the mechanical inconvenience of having filled the nasal passages with water. It frequently requires much gasping and several enormously exaggerated depressions of the floor of the mouth as if an attempt were being made to draw an obstruction from the nasal passages before finally unimpeded aërial respiration through these is reëstablished.

With this difference between the respiratory habits of lunged and lungless forms clearly in mind we are ready for the explanation of the part which the naso-labial groove and glands play in making possible the immediate resumption of aërial respiration after its temporary interruption by submersion in water.

When the nares are closed, there remains, externally, a slight depression, the floor of which is formed by the fold which closes the orifice. When the snout is thrust out of water there is a tendency, as the water drains from the surface of the head, for each nasal depression to retain some of it. Thus it would be expected that the opening of the naris for the resumption of aërial respiration would allow this water to enter the nasal passage, especially as the depression of the floor of the mouth in the act of inhaling would draw in the water first of all. As a matter of fact, however, nothing of the sort occurs. When the snout is thrust out of water, the accumulation of the fluid in the nasal depression is only momentary. With the opening of the naris through the drawing back of the crescentic fold, the last vestige of this drop of water disappears; and, although the whole surrounding skin is wet, the nasal depression and the naso-labial

[1] In a previous article on the Ypsiloid Apparatus of Urodeles (BIOLOGICAL BULLETIN, Vol. X., No. 6), I have shown that this species, which has been erroneously supposed to be lungless, really has well developed, functional lungs. In the same article is given also a more complete account of the respiratory habits of the lunged salamanders.

groove may be seen, by the aid of a lens, to be absolutely free from water which, moreover, *recedes slightly from the border of both naris and groove.* Although the general mechanical process by which the naris is thus quickly freed from water is obviously through the medium of the groove, the absolute determination, experimentally, of the physical principles involved in the performance of the function of the groove and glands proved somewhat difficult. The chief obstacles lie in the fact that the parts concerned are so extremely small that the experiments must be made under a lens of considerable magnifying power; while the animals themselves, which, of course, must be in their normal, active condition, are extremely agile and object strenuously to the experimenting process. The most effective device was found to be that of holding the specimen wrapped in a slightly moistened handkerchief with only the head protruding. Specimens handled in this way, however, soon become too much dried to react normally and must frequently be returned to the terrarium and a fresh specimen taken. Although many experiments were made, with varying degrees of success, the following set of experiments presents all of the facts from which the final conclusions were drawn. These experiments or slight variations of them were repeated many times and with different individuals, always with virtually the same results.

Experiment 1. — The head of the specimen was thoroughly dried by means of absorbent paper. The nares were open and normal aërial respiration was taking place. A small glass tube, one end of which was drawn out to a fine capillary point, was partly filled with water. By blowing into the larger end of the tube a tiny drop could be forced out through the capillary end. This drop was deposited on one of the nares, the object being to get a drop small enough to exactly fill the nasal depression. The result was the immediate closure of the naris and a subsequent immediate reopening, since the water disappeared at once. The experiment was repeated with a drop of diluted India ink substituted for the water. At once the groove became filled with the ink, which accumulated in a little drop at the labial end where it flowed off over the surface of the skin. The naris again reopened immediately, completely free from the fluid. The mouth was

then forced open and the region about the posterior nares examined to see if by any chance the ink had entered the naris. No trace of it was found, however, anywhere in the mouth cavity. This portion of the experiment shows, what of course the experiment with water had failed to show conclusively, that *the groove is the sole channel through which the fluid escapes from the naris.* The groove, moreover, retained a tiny line of the ink after the naris was free from it, and the ink remained there until a subsequent application of water to the naris resulted in the removal of the ink as the water took its place in the groove.

Experiment 2. — The head of the specimen used in this experiment was wet by allowing water to flow over it from a pipette. When the nares were opened and normal aërial respiration was established, the attempt was made to place a small drop of ink in the nasal depression. This proved difficult because the film of water which covered the surrounding area attracted the drop and the ink would flow off over the surface of the skin instead of entering the nasal depression. When finally a drop was successfully placed upon the naris, there was an immediate appearance of a line of ink filling the groove and escaping from its lower end. After a moment the naris opened as before, wholly free from the fluid. This experiment not only corroborates the conclusions drawn from the first experiment as to the function of the groove, but shows that *under the usual moist condition of the skin there is a tendency for a small drop of water falling upon the snout not to flood the nasal depression at all but to mingle with the general moisture covering the skin and to flow off over the surface.* This tendency is referable to that condition of the edge of the naris which causes the water to recede slightly from the orifice while it freely wets the neighboring skin and remains as a thin film upon its surface.

Experiment 3. — The head of the specimen was dried and a drop of ink was applied to the edge of the lip at the labial end of the groove. The groove at once filled with ink which extended *up* toward the naris. Upon reaching it, however, it produced no interference with respiration for it merely filled the wrinkles in the external surface of the crescentic fold which was drawn back to the outer edge of the orifice. *The experiment*

thus demonstrated with absolute certainly the capillary action of the groove and at the same time showed that *the relation of the groove to the fold renders it impossible for the naris to become flooded through this capillary action of the groove* even when the lower end of the latter is in the water, a common occurrence under normal conditions.

Experiment 4. — The head of the specimen was thoroughly dried with absorbent paper and, when normal respiration through the open nares was in progress, a thin film of olive oil in which lamp black was suspended was quickly spread over the whole surface of the head. There was the customary opening of the naris by the withdrawal of the crescentic fold, but *this was unaccompanied by the usual receding of the fluid from the edge of the naris and the draining off of the naris through the groove.* The groove seemed wholly obliterated by the oil, which formed a continuous film over it. A line of lamp black could, however, be seen slowly settling to the bottom of the groove, showing that the oil had entered it. The oil extended to the very edge of the nasal orifice and with each often repeated closure of the naris the nasal depression could be seen to fill again with the oil. The animal made frequent efforts to rub off the oil and finally being unsuccessful in freeing the nares of the oil it slightly opened its mouth and attempted to resume bucco-pharyngeal respiration through this new channel. Upon examining the interior of the mouth I could see that the black oil had passed through the posterior nares and had entered the mouth.

This experiment showed that the condition of the skin bordering the naris and groove, although such as to slightly repel water, did not have this effect upon the oil which could not, therefore, be drained from the naris but overflowed its edges and entered the nasal passages. This indicates that *the secretion of the naso-labial glands mixes with oil but repels water and that it is essential to the successful performance of the function of the groove.*

Experiment 5. — In this experiment a drop of water in which carmine was suspended was placed upon the naris. The particles of carmine were evidently too large for the groove, since there was no filling of the groove by the fluid. After a moment, however, there was a sudden disappearance of the larger part of

the drop. Evidently the crescentic fold had been withdrawn as usual but the cessation of the usual function of the groove had resulted in the fluid entering the naris. This inference was proved correct by an examination of the interior of the mouth in which the carmine was found.

In the light of these experiments the process of freeing the nasal depression from water may be explained as follows:

When, after the excess of water has flowed off from the general surface of the head, a small amount is left in the nasal depression, its surface tension is too great to allow its removal merely in obedience to gravitation. There is, therefore, a most delicately adjusted combination of devices by means of which gravitation is aided and the rapid removal of the water from the naris is effected. These are (1) the capillarity of the groove which is, especially at its nasal end, very narrow and deep; (2) the continuity through the medium of the groove, of the water in the nasal depression with the water at the labial end of the groove, even though this may be merely a larger drop which has accumulated on the surface of the lip; (3) the final impetus to flow down the groove, given by the wrinkling back of the crescentic fold in the process of opening the naris. It is impossible to say which of these factors is of chief importance. When the amount of water is very small, probably either the first or the last alone would suffice. With a larger amount of water the deeper portion of the groove quickly fills by capillary attraction and then its outer, wider portion probably serves merely as a gutter down which the water flows by gravitation. That the capillarity of the groove, working in the reverse direction, does not prove a disadvantage is due to the fact that the groove is continuous with the external surface of the crescentic fold which is, therefore, the only part affected.

Although the nature of the secretion of the glands has not been chemically tested, their function is obvious. The only portion of the head over which water does not flow freely and from which water seems in fact to be repelled is the region immediately surrounding the naris and the mesial border of the groove. In these regions microscopic sections show a noticeable lack of the large acinous glands of the skin, the secretion of which readily mixes

with water. These regions, on the other hand, are in the immediate vicinity of the orifices of the naso-labial glands. The motions of the crescentic fold are observable not only in opening and closing the naris, but to a lesser degree as frequent slight fluctuations. Undoubtedly these not only stimulate the flow of the secretions of the two large glands associated with the naris [cf. Bruner ('01)], but also effect the spreading of the secretion around the margin of the orifice. Furthermore, the orifices of the glands which are associated with the groove are elevated above the groove by their location upon papillæ. Thus while their secretion is discharged along the edge of the groove, the groove itself is kept wholly free from it. This is a necessary provision since because of the power of the secretion to repel water, it would, if it entered the groove itself, destroy its capillary power to remove water from the nasal depression. The nature of the secretion is probably such that it spreads easily upon the surface of the skin and thus effects the repulsion of water from the margin of the naris and groove. This function of the secretion is very important in two ways, first, it serves as a means of preventing the frequent flooding of the nasal depression from contact with drops of dew, for example; and, second, after the flooding of the naris has occurred, it acts as a means for quickly shutting off the water which lodges in the nasal depression and groove from the film of water upon the surrounding surface of the skin and thus makes it possible for the groove to remove the residue of water from the nasal depression. The large size of these glands proves that their function is one of great importance.

As to the purpose of so delicately adjusted and efficient a mechanism for quickly freeing the nares from water, one can only conjecture. It is undoubtedly connected with the apparently universal habit among the *Plethodontidæ* and *Desmognathidæ* of maintaining a dry condition of the nasal passages. That the ability to reëstablish aërial respiration so promptly is primarily because of the importance for that particular mode of respiration seems, however, to be disproved by the experiment above cited in which specimens lived for many days immersed in water with no access to air. Neither was it found in any way injurious to artificially fill both the nasal passage and the whole mouth cavity with

water, an experiment which I made by blowing water in through a capillary tube inserted into the naris. The individual thus treated merely experienced considerable inconvenience for a few minutes in his efforts to remove the water from the nasal passages, an end which he finally accomplished by swallowing forcibly. After a few minutes he was breathing air as usual.

I am convinced, however, that the respiratory functions of lungless salamanders are not as yet fully understood and it is very probable that further experimentation may reveal conditions under which the ability to promptly resume aërial respiration is a matter of great importance.

Another possibility suggests itself as an explanation of the need for the naso-labial apparatus. These animals are nocturnal in their activities and it is probable that in their search for food they are largely dependent upon the sense of smell. If so, their successful search would be much interferred with by even a temporary cessation of bucco-pharyngeal respiration, since it is by means of this act that the odors reach the nasal epithelium. In view of this supposition a device for quickly freeing the nares of water would be of the greatest importance, since in these nocturnal excursions the animal must be constantly wetting the head with drops of dew.

The question naturally arises as to the conditions in the one species, *Salamandrina perspicillata*, which is known to be lungless and yet to possess no naso-labial groove. I have unfortunately been unable to obtain living specimens of this European form. It is said, however, to have a bucco-pharyngeal *aquatic* respiration and in that case would not be expected to have a highly specialized apparatus for maintaining a dry condition of the nasal passages. In all events, its lungless state was acquired more recently than that of the *Plethodontidæ* and *Desmognathidæ* and quite independently of them, and may have been the result of very different conditions.

Throughout the investigations involved in this article I have been much helped by the advice and criticism of Dr. H. H. Wilder, and I wish here to express my gratitude to him for the assistance thus rendered to me.

CONCLUSIONS.

1. The naso-labial groove is a characteristic of the adult stage of the *Plethodontidæ* and *Desmognathidæ*.

2. Associated with the naso-labial groove is a system of glands which lie beneath the skin, and, in the main, external to all skeletal structures and open upon the border of the naris and groove.

3. These glands are homologous with the external nasal glands of other *Salamandrida* and of the *Anura*, but in the lungless families certain of them attain an enormous development.

4. The function of the naso-labial apparatus is to quickly remove from the nasal depression the small quantity of water which collects there when the head has been flooded with water. The object of this prompt removal of water is to permit an immediate resumption of aërial bucco-pharyngeal respiration after its temporary interruption by immersion in water.

5. The function of the glands is to guard both the naris and the groove against a continual overflow of water from the neighboring wet surface of the skin. The secretion is of a nature to repel water and by its agency water lodged in the nasal depression is cut off from that on the surrounding surface and can therefore be removed through the groove.

6. The groove performs its function partly through its capillary action, thus overcoming the surface tension when the quantity of water to be removed is very small; and partly merely as a gutter permitting the flow of the fluid from the naris to the lip. In its function it is assisted by the motion of the crescentic fold of skin which is drawn across the naris to close it against the entrance of water. The withdrawal of this fold tends to push the water out of the depression into the groove.

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NOTES ON THE MORPHOLOGY AND DEVELOPMENT OF TWO SPECIES OF EUDENDRIUM.[1]

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The observations presented here were made while describing and cataloguing hydroids collected at Bermuda during the summer of 1903. They have to do with two species of the genus *Eudendrium*. In one of these, *E. ramosum*, the development of the female gonophore is outlined, since no such account, so far as I am aware, has yet been given for any member of the genus. To a review of the histological conditions found in *E. hargitti* is added a description of its oögenesis which is unique for the genus in that the egg grows by the bodily absorption of entoderm cells. *Eudendrium hargitti* is here described for the first time.

To those who are acquainted with the contributions of Professor Charles W. Hargitt to our knowledge of the genus, the significance of the specific name will be evident. I wish to acknowledge my personal obligation to Professor Hargitt for suggestions and criticisms relating to my work.

Development of the Female Gonophore of Eudendrium ramosum.

The egg cells destined for the female gonophore here, as in all members of this genus, have their origin in the unfascicled branches of the mature colony and pass distally to the region where the future gonophore is to develop. They are first distinguished from epithelial cells by their deeply staining cytoplasm and large nuclei. They are found scattered along through the entoderm of the branches and pedicels and are most abundant close to hydranths bearing young gonophores. They also may be seen in the entoderm of hydranths which as yet show no external signs of gonophores.

Conditions point clearly to their migration up the branches. They show an increase in size proportional to their proximity to

[1] Contributions from the Zoölogical Laboratory of Syracuse University.

the end of the branch. Near the terminal hydranth they are so large as to crowd the neighboring entoderm cells, bulging the entoderm inward and mesoglœa outward. Their shape and position are such as most easily to force their way along the branch. They are flattened between mesoglœa and entoderm and their thin edges are inserted, wedge-like, between the two layers. In the fixed material I could find no indication of pseudopods. The entoderm cells about them are compressed and distorted in the way we would expect if the egg should move along under them.

There are two types of female gonophores differing so much in size and position that one might reasonably consider them entirely independent, were it not for the evidence of their common origin. In one type the outline of the spherical egg can be readily seen. An entodermal thickening passes half way round it extending from its attachment to hydranth, up over the top, to a point opposite its origin (Fig. 1). Here the ridge divides into a pair of branches which form open coils on opposite sides of the egg. Other gonophores have a distinctly different position in the colony. They are ovoid and the egg is largely hidden by a sheath of ectoderm and entoderm which on account of its development may be looked upon as a straight spadix (Fig. 2). The two kinds have been termed orthospadiceous and streptospadiceous according as the spadix is straight or coiled.

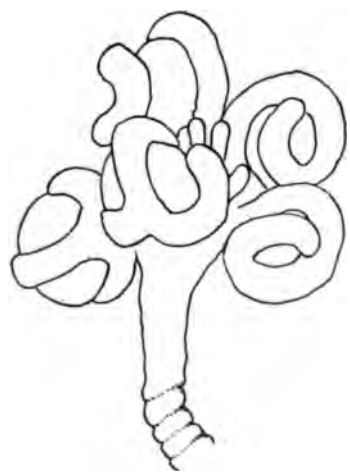

FIG. 1.

Streptospadiceous gonophores form whorls around the bodies of hydranths. Two, four, five or six gonophores are usually associated. Either the single terminal hydranth of a branchlet bears the gonophores or several succeeding hydranths may do so. The appearance of a gonophore terminates any further growth of a branchlet.

The earliest indication of the gonophore is an outwardly directed pocket of ectoderm and mesoglœa containing the egg

(Fig. 3). This is produced by the active proliferation of the ectoderm adjoining the egg. For a time the cells differ little from those of the hydranth ectoderm. Soon the entoderm just above the egg begins to form a blunt tube of columnar cells opening into the enteric cavity. The end grows outward between egg and mesoglœa. Growth of the base aids in carrying the egg out from the general surface of the hydranth (Fig. 4.) Continued elongation brings the end of the tube over the top of the egg and down the outside where it divides sending a branch inward and upward on either side to form the coil. Viewed from the side the spadix now looks like a spiral of one and a half turns. Linear growth of the spadix is nearly complete when the egg has one third of its mature diameter. It stands out from the surface at first, but in time becomes flattened against the egg.

FIG. 2.

The ectoderm undergoes a gradual change to a thin tough sheath covered by a tenuous perisarc. It passes over the spadix and elsewhere is separated from the egg by the mesoglœa only. Together with the base of the spadix, it forms the stalk of the gonophore.

While the spadix is developing the egg rounds out into a sphere and rapidly enlarges. Its increase in bulk is due to the formation of large granules of deutoplasm. Weismann, '81[1], describes a contraction and expansion of the spadix in the living gonophore of *Coryne pusilla* which he thinks functions to move the nutritive fluid, used by the growing egg. Variations in the diameter of spadix lumen of *E. ramosum* suggest that the same motion occurs in it. There can be no doubt that the egg absorbs nourishment through the walls of the spadix, as has been pointed out by others. Dr. Hargitt has suggested that the flattening of the spadix is due to the absorption by the egg of the contents of its cells.

Orthospadiceous gonophores occur near the proximal parts of the colony. When associated with the other type they are below them on the branch. Only near the base of the colony do they occupy the tips of the branches. They are preceded by the streptospadiceous gonophores in time of development, yet in mature colonies exceed them in abundance. A dozen clusters may occur on one branch and such branches be so numerous at the base of the colony as to form a considerable mass of gonophores.

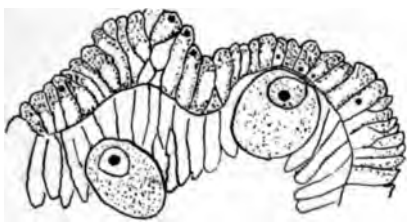

FIG. 3.

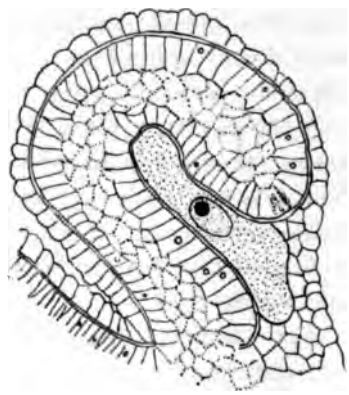

FIG. 4.

Five to seven gonophores usually form a cluster though any number from two to nine is not rare. They are attached along and around an annulated axis several times their length and of greater diameter than a hydranth pedicel. There is no definite form of arrangement. The base of one extends down part way below the top of another. They are generally associated as closely as their fusion on one side with the stem will permit. A division into a distal and proximal group at times is indicated by a slight free space on the axis.

In places where the two types are contiguous the transitional clusters may be present. As we would expect, they consist of orthospadiceous clusters below on a pedicel, with streptospadiceous, above on a hydranth. It is not an unusual thing to find gonophores that combine the two kinds of spadices.

The course of development in the orthospadiceous gonophore is much like that of the other type. The egg in the entoderm

of the hydranth lies, from the first, in contact with the mesoglœa. The ectoderm forms a pocket about it but does not separate it on its inner side from the body wall. While it is increasing in size, two entodermal processes pass out around it, the one from above and the other from below; of these the latter is the longest. They meet on the outside near the top. As they approach, a cup grows upward covering the surface between the lower process and the stem and sending ahead on either side a short projection. Growth ceases when two small spaces are left at the top, each of which is nearly cut in two by one of the lateral projections.

As the gonophore matures, the ectoderm secretes a perisarc which remains after the contents disappear. The ectoderm, itself, also becomes more opaque and tough. I find no peculiarity in the development of the egg. As one side is close to the enteric cavity there is no need of a spadix tube to supply nourishment. The entoderm of the gonophore is thrown into folds so disposed that the tissue is thin in the vicinity of the egg.

It seems probable that one type of gonophore arose from the other since intermediate gonophores are sometimes found. Conditions of occurrence and details of structure point to the streptospadiceous as the ancestral form. It is borne on the hydranth like the typical hydromedusan gonophore or medusa bud. The streptospadiceous gonophore is borne on a branchlet or pedicel. It grows on the younger part of the colony, is first to appear in the individual colony and so may well have been the first in the history of the species. The structure of gonophores and gonophore clusters point to the same conclusion. They show further that the process was one of simplification and involved a decrease in the time and material used in the developing gonophore. If we imagine the end of the coiled spadix to be directed upward and the whole structure to flatten down on the egg, thus fusing into a continuous cup whose rim is near the end of the spadix, we will have a spadix of the orthospadiceous type. Further simplifying of the gonophore by removing its pedicel and allowing it to sink into the stem for a third of its diameter will make it entirely orthospadiceous. Many of the large orthospadiceous clusters of six or eight gonophores are divided into a proximal and distal group by a small free space midway on the pedicel.

The comparison of a mixed cluster with these suggests strongly that the distal parts of their pedicels are homologous to hydranths and the proximal part to hydranth pedicels. A mixed cluster consists of a hydranth body bearing streptospadiceous gonophores and its pedicel bearing orthospadiceous gonophores. If its hydranth becomes reduced to a pedicel and its gonophores are simplified to the orthospadiceous condition we will have a structure corresponding exactly to the double orthospadiceous cluster described above both in form and number of parts. The division between the proximal and distal group has disappeared in half of the large clusters. It would be mere speculation to homologize the small clusters with the double ones. If the distal orthospadiceous gonophores of the clusters arose from the streptospadiceous type it seems further probable that the orthospadiceous gonophores on the pedicel arose from streptospadiceous pedicel-gonophores which in their turn are phylogenetically younger than those on the hydranth body.

General Morphology of Eudendrium hargitti.

The hydranths are of a bright reddish-brown, the stem of varying shades of brown. Stem unfascicled, colony twenty to fifty millimeters long, becoming nearly transparent near the extremities. Branches straight, few, nearly parallel to the main stem, distributed irregularly, joining the stem by an abrupt bend. Annulations at base of colony and branches, occasionally elsewhere. Hydranth most deeply colored at base of hypostome, tentacles from thirty-five to forty-five, in contraction forming two closely appressed rows, hypostome very mobile, contracting into a shallow cup or extending to a length greater than the hydranth body. Some hydranths provided with a groove near their base containing gland and thread cells.

Histology.

The majority of the histological details of this hydroid correspond with descriptions of other forms contributed by various authors. There are, notwithstanding, a sufficient number of unique conditions to make a general review not without value.

Hypostome. — One marked peculiarity of the hydranth is the unusual extent to which the hypostome may be expanded or contracted. As a result we have interesting conditions in the muscle processes and the thread cells. A glandular ring frequently found near the base of the hydranth is in part like a structure described by Weismann for two species of the genus *Eudendrium*, and not elsewhere recorded (Fig. 6).

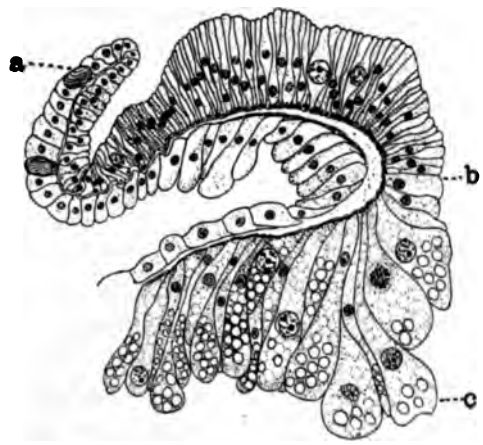

FIG. 5.

The ectoderm cells of the hypostome vary greatly in form during different phases of the contraction or expansion of the hypostome. They appear as pavement cells in the extended organ. If extremely contracted they are not only columnar, but the free ends bulge outward. Their cytoplasm is granular and often vacuolated. The nucleus, like that of entoderm, is large, with a nucleolus and at times a chromatin reticulum. Thread cells occur in a definite ring below the mouth (Fig. 5). Since the tentacles are largely destitute of them, it is apparent that the hypostome has usurped part of the protective function which belongs to the tentacles in most gymnoblastic hydroids. The entoderm of the hypostome is thicker than the ectoderm and consists of more diversified elements. The cells are cubical or columnar, depending upon the degree of extension of the hypostome. Greenish-yellow pigment granules occur here and there

through the tissue. Glandular activity is evinced in some cells by the deeply staining granular contents which may be seen discharging into the enteric cavity.

Gland cells of another type, most abundant near the mouth, present a striking appearance in the early stages of secretion. Small deeply staining granules form a single layer parallel to, or touching the cell wall near the free end. They are arranged with perfect regularity at equal distances and present a sieve-like effect. Certain hydranth cells which otherwise appear the same are full of the granules. I consider that the two types represent earlier and later stages of secretion. In any one hydranth the cells seem to go through the stage of secretion nearly at the same time. The nuclei are smaller than in any other cells of the hydranth.

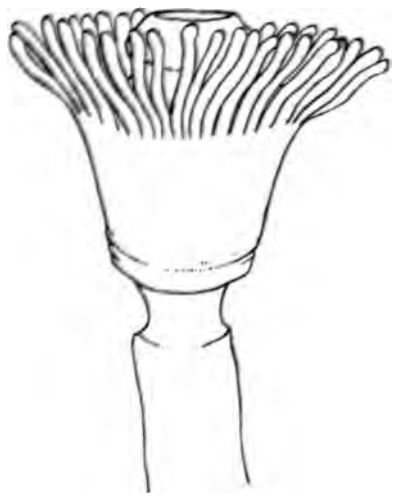

FIG. 6.

Body. — The entoderm of the body, as usual, contains many glandular digestive cells. They are very large about the internal opening of the hypostome and project downward so far as to form a thick ring which may nearly fill the cavity of a contracted hydranth. Some of them are granular and deeply staining. Others are vacuolated and often found in the act of discharging their contents into the enteric cavity. Since transitional cells are present it is probable that the first are earlier stages of secretion of a single kind of cell. May, '03, describes similar gland cells in *Corymorpha pendula*.

Spherical masses as large as nuclei appear in the gland cells and are given a saffron tint by Ehrlich's triple stain. Their interior is generally homogeneous but may contain a sphere suggestive of a nucleolus. No chromatin is found in them. They are frequently given off by gland cells and appear to be the remains of nuclei.

Pigment granules are present in all parts of the entoderm. They are most abundant in the gland ring. They are reddish-brown in living tissue and greenish-yellow and non-staining in alcoholic and formalin material. Lendenfeld, '83, found pigment in the entoderm of the hydranth of *Eucopella campanularia* and along the radial canals and about the egg of the medusa. Since it occurs in regions of greatest metabolism he concludes that it is the result of metabolism and therefore an excretory product. A similar point of view is taken by Hargitt, '04, in an extended consideration of the problem of coloring among Cœlenterata. In *E. hargitti* there is an association between the pigment and gland cells. It is most abundant in the gland ring where secretion is most active. It is found at the interior end of the cells like other secretory products. I found a few cells in the act of discharging it. Since it is as far as possible from the exterior, its position is plainly least suited for producing a coloring of the animal. Small amounts also occur in the pedicels and branchlets where the perisarc may preclude any color being seen from the exterior. Since the habits of the animal do not point to any advantage from its coloration I am inclined to think that the pigment has chiefly, if not entirely, its reason for existence in the metabolism of the animal. Between the gland cells the entoderm contains shorter, less deeply staining columnar cells. They become more abundant toward the base of the hydranth. Gland cells exceed the others on the folds of entoderm resulting from contraction of the hydranth.

Fibrous muscle processes from the bases of the ectoderm and entoderm cells, similar to those found by Kleinenberg in *Hydra*, occur here. They stain readily in iron hæmatoxylin and are so abundant as to offer an unusually good opportunity for study. They extend longitudinally along the mesoglœa in the ectoderm. In favorable sections single fibers may be traced the entire length of the hypostome. They are most abundant in the entoderm where they take a circular course. In a contracted hypostome they form a thick zone next the mesoglœa. Here it is easy to see that the fibrous process of the cell consists of a thin sheath of protoplasm surrounding a deeply staining filament. Longitudinal ectoderm and circular entoderm fibers occur to less numbers in all parts of the hydranth body and in the tentacles.

Tentacle. — The few thread cells of the tentacles occur for the most part in the thickened ectodermal tip. There are no external ridges as in *E. ramosum.* The entoderm cells appear polygonal in a cross section of the tentacle and form regular transverse layers of uniform thickness. The nuclei are so large as to appear to fill the interior of contracted tentacles. Longitudinal contractile fibers extend along the exterior of the thin mesoglœa. Their distal ends can sometimes be traced out among the ectoderm cells, though I could not demonstrate any connection with them.

Gland Ring. — Around the base of the hydranth the ectoderm is modified to form the gland ring mentioned previously (Fig. 6) The entoderm does not take any part in its structure. Weismann's description, '81[2], of similar rings refers to *E. racemosum* and *E. capillare.* He states that it consists of two ridges surrounding the base of the hydranth, separated by a narrow groove. The upper one has a varying number of thread cells; the lower is glandular and may be seen discharging its product which cements together a ring of débris about the base of the hydranth.

Above the upper ring occurs a muscular organ which Weismann terms a cnidophore. It is much like a large tentacle thickly beset with thread cells. The combined structure occurs only occasionally and more often on male than female colonies. He thinks the cnidophore a weapon developed as a protection against some especial enemy. The hydranth of *E. hargitti* very often possesses a double ring, but I have not found the cnidophore. The degree of development of the ring is extremely variable. At times it is plain to the unaided eye, again not readily detected even with sectioning and staining. Weismann does not describe gland cells in the upper ring. They are plentiful in the Bermuda form wherever it is devoid of thread cells. The distinctly glandular tissue is two or three cells thick and changes gradually into unmodified ectoderm. As noted by Weismann, the length of these cells is considerable, as they extend from the top of the ridges to the mesoglœa. Their cytoplasm is deeply staining and granular. The free end swells outward as in other gland cells. Often the upper ring presses down on the other so that the groove opens at a lower level than its interior. The bottom is formed from the extended bases of the gland cells.

The function of the upper ring of thread cells is probably protective, as we can hardly conceive of any offensive use in this place. The object of the glandular tissue of the two rings as well as of the groove seems obscure. The occurrence of rings without cnidophores goes to confirm Weismann's statement that cnidophores and rings have independent functions.

Pedicel and Stem. — There is little unusual in the histology of the pedicel and stem. The narrow neck connecting hydranth and pedicel consists of typical cambium tissue as described by Jickeli, '82; Seeliger, '94, and others. The enteric cavity is much constricted. The entoderm consists of brightly staining cells which appear in more vigorous condition than much of the tissue below them. The cell borders are well marked. Active gland cells occur here and there.

The ectoderm of the cœnosarc is first affected by degenerative changes of all the tissues of the colony. Large masses of thread cells occur in it, when thus affected, and they are in all stages of development. The youngest appear as small refractile bodies in cells full of deeply staining granular cytoplasm. As they grow larger a deeply staining rod appears in the axis of the body. It develops into a loop, and then into a coil of about ten loops. At this stage the membrane of the parent cell is filled by the structure and the shrunken nucleus is crowded between it and the membrane.

Weismann, '81[2], described slow peristaltic contractions of the cœnosarcal column of hydroids as well as amœboid movements of ectodermal processes along the inside of the perisarc. When a cover glass was pressed down over a freshly cut portion of a branch some processes were seen to withdraw from the perisarc and others to reach out to it. I find a condition of the cœnosarc very suggestive of such movements. In most branches the ectoderm is attached to the perisarc in but few places and is elsewhere contracted in varying degree. Doubtless a certain amount of contraction also results from the action of the killing fluid.

Bands of mesogloea sometimes are found extending to the perisarc of pedicels and branches. The ectoderm reaches out and around the band to its point of attachment, thinning to a mere film which passes between it and the perisarc (Fig. 7).

The structure seems to have arisen through the mesoglœa in some way becoming attached to the perisarc without destroying the continuity of the cuticular border of the ectoderm cells. This could most easily take place during some time of expansion of the cœnosarc while the perisarc and mesoglœa were near each other. When contraction occurs for any reason, such as the killing of the tissue, the ectoderm would be drawn tensely up around the mesoglœal connective by the cuticular film.

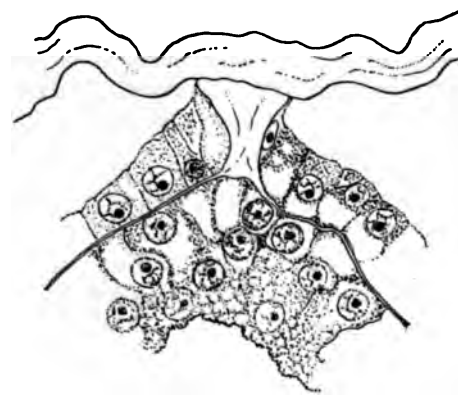

FIG. 7.

Weismann found a very similar or perhaps an identical condition in a species of *Eudendrium*. He says he found the cœnosarc near the band pulled out to form a pocket to the enteric cavity lined with glandular cells differing from the entoderm and forming a secretory organ. He does not describe a pulling out of the ectoderm to the point of attachment of band and perisarc.

I found a pocket in the enteric cavity resulting from the contraction of the cœnosarc. It seems to be a mechanical necessity that one be formed in the region held close to the perisarc by the band. When the cœnosarc is expanded there is no pocket. A few gland cells occur in the pocket but no more than elsewhere in the entoderm. It is plain that there is not a distinct glandular organ here and I doubt whether the structure is other than incidental. In the expansion and contraction of the cœnosarc it seems reasonable that the apparently viscid mesoglœa should become attached to the perisarc, especially in places where the entoderm has more or less deteriorated.

There is no peculiarity in the cœnosarcal ectoderm. It is columnar and has gland cells occurring singly or in groups similar in type to those of the hydranth body. As we pass into the larger branches it comes to consist of several layers as in other hydroids. The perisarc of young branches is generally homogenous. It shows lamination as it matures and often has dark interior layer.

Oögenesis.

Especial interest attaches to the development of the egg, because we have a condition hitherto undiscovered in any member of the genus. It is the absorption of the neighboring cells during a period of its growth. The manner in which this take place does not entirely correspond with that of any other species of hydroid whose oögenesis has been investigated. I confine this description to the streptospadiceous gonophore as very few young orthospadiceous gonophores were found. From examination of later stages of the latter, I am convinced that there is no marked difference in their method of development.

Origin and Migration. — The interest which has formerly centered in the question of the origin and migration of the eggs of Coelenterata and especially *Eudendrium* has resulted in a definite knowledge of the facts in many of the common species. Weismann, '71, and Hargitt, '04[1], have shown that the ova of *E. ramosum* originate in the ectoderm. Hargitt has since announced that in *E. tenue* there is a similar origin. In *E. racemosum* and *E. dispar* he found eggs in both layers, but the ectoderm was plainly the place of origin.

Because there has been controversy as to the parent tissue in *E. ramosum*, I examined several thousand sections from the branches and pedicels of *E. hargitti*, that any early migration of the egg from one layer to another should not escape me. Without an exception ova were found in the ectoderm. I was not able to find an ovum as small as an ectoderm cell because the ectoderm of the branches of mature colonies was in such poor condition. The smallest ova were about three times their bulk (Fig. 8). Since the eggs lie against the mesoglœa and push the adjoining cells to either side by their growth, it is beyond doubt that they have originated there. They occur singly.

Since the egg is found in the entoderm of the hydranth and is always in the ectoderm while in the pedicel, it is apparent that it must pass through the mesoglœa at the restricted neck which joins the hydranth and pedicel or in the lower part of the

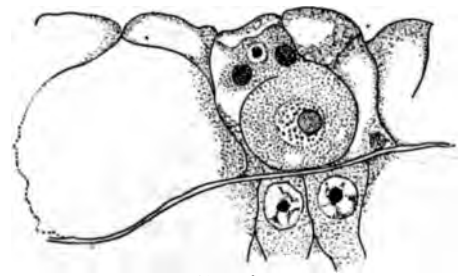

FIG. 8.

hydranth. It is probable that the passage is at the former place because not only is the wall very thin there but the egg on its arrival has often attained a diameter greater than the thickness of the wall. In a single preparation an egg was found in the ectoderm at the end of the pedicel. The mesoglœa at its anterior end was almost gone.

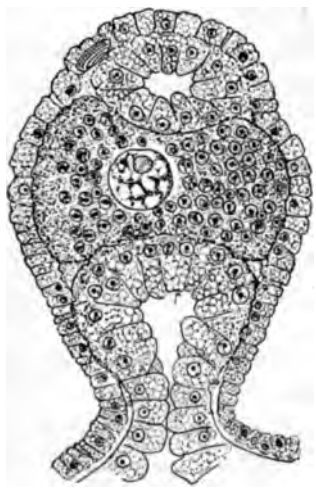

FIG. 9.

On its arrival in the hydranth the ovum is so large that it pushes the surrounding entoderm far out into the enteric cavity. The growth of the gonophore by the outpushing of the body layers, and the formation of the spadix duplicate the processes in *E. ramosum*. Since that has been already outlined it is not necessary to consider it here.

Growth of the Egg. — The smallest ova are dissimilar to the ectoderm cells in all details of structure. The cytoplasm is granular and deeply staining. It sometimes contains vacuoles which are most common near the periphery. The nucleus has a distinct membrane, a spherical nucleolus and a chromatin reticulum (Fig. 9).

During the stay of the ovum in the pedicel there is an occasional absorption of neighboring ectoderm cells. It is indicated by the presence of degenerating nuclei within the ovum. At first they differ little from the nucleus of an ectoderm cell. At a later stage the nuclear membrane disappears leaving a vacuole larger than the original nucleus and containing deeply staining chromatin rods surrounded by a non-staining watery substance. Similar nuclear degeneration may be traced more completely in the general ectoderm tissue up to the formation of the vacuole.

A more active cell absorption takes place when the egg reaches the hydranth (Fig. 10). As much as half of the egg membrane

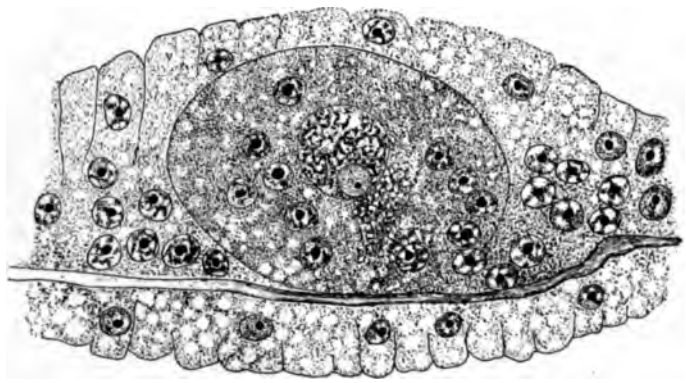

FIG. 10.

on one side may disappear in company with part of the walls of neighboring entoderm cells. A thin cuticular film stretching over the inner side of the egg separating it from the enteric cavity is all that remains of the walls of the nearest cells. The more distant cells retain the part of their wall farthest from the egg. There is no break in the continuity of the oöplasm and the fused cytoplasm of the entoderm cells. Nuclei are scattered about irregularly. Some occur definitely within the confines of the egg. As soon as the egg becomes enclosed in the gonophore it presents an unbroken periphery. When it has been carried away from the surface of the hydranth its nutriment comes by way of the spadix lumen which is a prolongation of the enteric cavity of the hydranth. Part of the columnar cells

constituting the spadix wall are in contact with the egg at one end since the spadix lies directly against the egg membrane. At their other end they extend for varying distances into the lumen producing a folded surface not unlike a digestive epithelium. It is probable that this large free surface is important in augmenting the absorption of food which is passed on to the egg (Fig. 11).

Two views have obtained as to the way in which the hydroid egg takes up the cells. Balfour's text-book of embryology, pub-

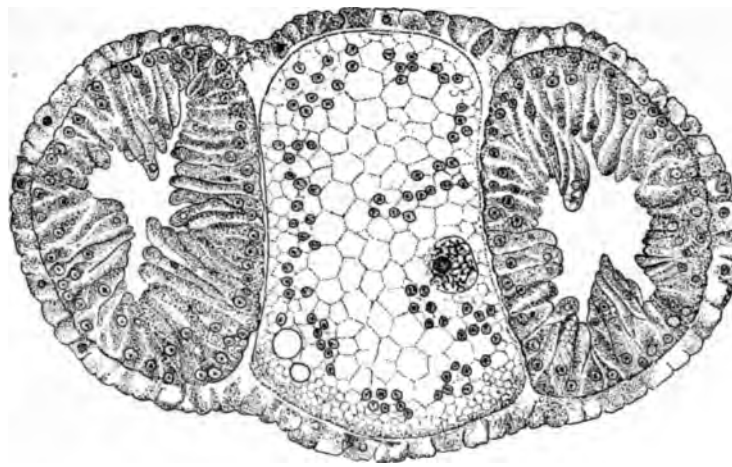

FIG. 11.

lished in 1879, states that they are amœboid and do so by putting up pseudopodia. Metchinkoff in 1887 described such a method in some medusæ. Tichomiroff in the same year found a condition in certain hydroid eggs which he terms an "amœboides Fressen." Smallwood, '99, saw indications of amœboid activities in the egg of *Pennaria tiarella*.

A different conception as to the process of absorption is found in Doflein's account, '96, of the development of the egg of *Tubularia larynx*. It is apparent from his discussion that he has studied the question carefully. He concludes that although eggs have processes like pseudopodia, there is no amœboid movement. Absorption occurs by the breaking down of the walls of the egg and adjacent cells to form a syncytium-like structure. When the

egg wall becomes defined again it includes the contents of other cells. Allen, '00, working on *Tubularia crocea* was led to Doflein's view, though the fact that the absorbed cell sometimes retains its outline in the egg is suggestive of amœboid activity.

The egg of *E. hargitti* is largely passive in the process of cell absorption. A syncytium-like structure is formed by the disappearance of the egg membrane in a limited region, and of the walls of adjoining entoderm cells. The border of the egg is, later, again defined, not by its activity so much as by the growth of the spadix which removes the egg from its former position. It is not surprising that the behavior of the egg finds an approximate parallel in *T. larynx* and *T. crocea*, when we consider that these species belong to a closely associated genus.

There has been a diversity of view as to the fate of the nuclei of cells absorbed by hydroid eggs as well as to the method of absorption. Ciamician, '79, applied the term "pseudozellen" to spherical bodies found in the egg of *T. mesembryanthemum* which he considered to be formed after the nuclei of the absorbed cells had disappeared. Brauer, '91, published the results of the study of the same species, in which he agreed with the conclusion of Ciamician as to the origin of the pseudo-cells. Also, in a contribution to the development of *Hydra*, he says that pseudo-cells are formed in the cytoplasm and are not degenerating nuclei.

The three papers already mentioned, by Doflein, Smallwood and Allen, described the nuclei of absorbed cells as undergoing divisions and degenerative changes characteristic of pseudo-cells. That name is therefore applied to them.

In this species, the history of the nuclei does not exactly correspond to any that have been outlined above but finds its closest parallel in the persisting of nuclei as pseudo-cells. I have not as much evidence as I would like regarding the absorption of nuclei by the egg near the place of its origin. This is due to the difficulty encountered in finding very young eggs. A considerable part of those I did find had nuclei of ectoderm cells, thus rendering it probable that there is an early stage of the egg when growth is expedited by the absorption of a few cells. For two reasons, I am convinced that the nuclei are absorbed before the egg leaves the pedicel; first, no extra nuclei were found in many

large eggs examined from the pedicel; second, as already stated, a nucleus was found in process of absorption in a small egg. As has been shown the period of most active absorption is during the stay in the wall of the hydranth. When the egg is first enclosed by the gonophore, nuclei are scattered thickly throughout the cytoplasm. As the deutoplasm is formed they are in part crowded toward the periphery. We might expect from the conditions in *Pennaria* and *Tubularia* to find the nuclei now passing through the characteristic changes of pseudo-cells, but nothing of the kind occurs. In eggs well on toward maturation, I detected no change in the nuclei. It would be of great interest to find whether the pseudo-cells are finally formed or whether the history of the nuclei is different from any yet described, in that they are gradually absorbed. The persistence of the nuclei during the maturation of the egg appears less strange when we consider that the ova grow very rapidly and that the period under consideration is but a comparatively few hours.

The results of the latest investigations of pseudo-cells seem to show that Brauer and Ciamician were wrong in the conception of their origin. If that be true, then the term rightly applies not only to the bodies found in the eggs of *T. larynx*, *T. crocea* and *P. tiarella*, but in *E. hargitti* as well, although we do not know whether the latter ever undergo like degenerative changes.

From the time of origin of the egg in the ectoderm until its enclosure in the gonophore the nuclear structure does not change though the size increases. As the gonophore approaches its mature proportions, the nucleus becomes excentric and may lose its membrane. I did not follow the phenomena of maturation farther.

Yolk granules appear in the egg while the gonophore is small. They are usually first developed around the central nucleus. In any case the periphery is the last to become filled with them. They enlarge and increase in number so as to take a polyhedral shape to mutual pressure. They are separated by a thin film of protoplasm. As in *E. ramosum*, the spadix becomes flattened upon the egg probably contributing its substance for nutrition.

Nourishment. — In connection with the study of the gonosome I found it of interest to compare the distribution of nourishment

in the young and sexually mature colonies as shown by their histological condition. In the former there is enough food not only for the hydranth and the rapidly growing cambium tissue at the ends of the pedicels, but for the small requirements of the cœnosarcal column down to the very base of the colony. In the latter, the rapid growth of the eggs must cause the absorption of a greater proportion by the hydranths and the pedicels of orthospadiceous gonophores. The tissue farthest removed from the food supply is the ectoderm of the cœnosarc. It is also least necessary to the economy of the colony at this period of its life. Accordingly we find it undergoing retrogressive change in mature colonies at the same time when gonophores are vigorously developing. The last part of the cœnosarc to cease its activity is the cambium tissue, close to the hydranths.

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A PECULIAR TYPE OF NEPHRIDIA IN NEMERTEANS.

WESLEY R. COE.

The excretory apparatus of the nemerteans has long been known to present great variation in form and structure in different members of the group. In many of the smaller and less highly specialized forms the apparatus presents a close similarity to that of the planarians and has evidently been derived from a similar type. In many species a profusely branching system of tubules lying in close proximity to the blood vessels connects with a single excretory pore on each side of the body, while in other forms the number of such efferent openings may be increased to the number of thirty or more on each side, as in some species of the genus *Lineus*. Although limited to the cephalic or a small portion of the esophageal region in some species, the system extends throughout the entire length of the body in other forms, as in the fresh-water *Stichostemma*. In a few species which have been carefully studied the system has not yet been discovered, in some cases perhaps owing to the difficulty of distinguishing the delicate nephridial tubules from the branches of the thin-walled blood vessels.

With the exception of certain species of the genus *Tæniosoma* (*Eupolia*) all nemerteans in which the relationships of the nephridial system are known agree in one important feature, namely, in that these excretory tubules, whatever their structure or position, open and discharge their contents directly upon the exterior of the body.

Several years ago Punnett,[1] in investigating the anatomy of *Tæniosoma quinquelineatum* (*Eupolia melanogramma* Punnett), found that this species possesses a considerable number of efferent nephridial ducts, some of which open on the exterior of the body, as usual, while others pass inward through the epithelium

[1] Punnett, R. C., "On a Collection of Nemerteans from Singapore," *Quart. Journ. Micr. Sci.*, Vol. 44, p. 116, 1900.

of the esophagus and open into the lumen of this portion of the alimentary canal.

In a recent study of a species of the same genus (*Tæniosoma cingulatum* Coe) from the Hawaiian Islands, I have found a very similar condition.[1] In this species the nephridial ducts are profusely branched, and extend through the posterior three fifths of the esophageal region. The branches are of rather large size and are situated on the lateral walls of the esophageal blood lacunæ, in close contact with the epithelial lining of the blood spaces (Figs. 1, 2, 3).

The total number of efferent ducts is between ten and thirty on

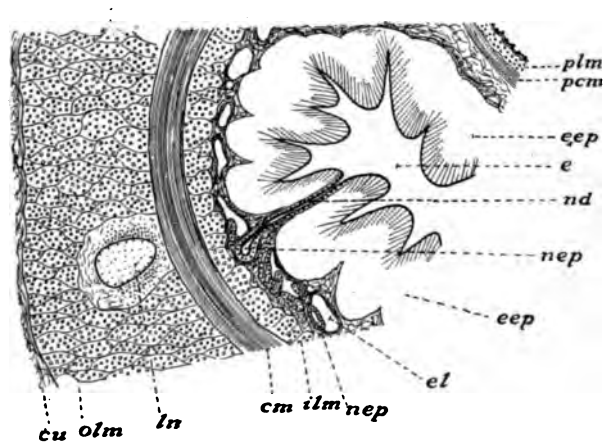

FIG. 1. *Tæniosoma cingulatum.* Portion of transverse section of body through esophageal region, showing efferent duct (*nd*) leading from a large nephridial canal (*nep*) through esophageal epithelium (*eep*) to lumen of esophagus (*e*); *el*, esophageal blood lacuna; *cu*, cutis; *olm* and *ilm*, outer and inner longitudinal muscular layers respectively; *cm*, circular muscular layer; *ln*, lateral nerve; *plm* and *pcm*, longitudinal and circular muscles of proboscis sheath. × 75.

each side of the body, and those which open into the esophagus are interspersed irregularly with those opening upon the external surface of the body. The average position of those opening externally, however, is somewhat more anterior than that of those leading to the esophagus. The latter are more numerous than the former, and are much the more conspicuous. The appear-

[1] Coe, W. R., "Nemerteans of the Hawaiian Islands, Collected by the Steamer Albatross in 1902," Bull. U. S. Fish Com., 1903, p. 984, 1906.

ance would indicate that the ducts opening into the esophagus furnish the principal course for the discharge of the excretory products, while those which pass externally are little more than vestiges of the primitive condition, for many of the latter are incomplete and fail to reach the surface.

The efferent ducts which open into the esophagus exhibit a distinct, though thin-walled, tube passing directly from one of the larger nephridial canals to the surface of the epithelium lining the esophagus (Figs. 1, 2, 3). The presence of a distinct lumen throughout the whole length of some of the ducts does not leave the slightest doubt as to the actual communication between the nephridial canals and the lumen of the esophagus.

In all cases the openings of the nephridial ducts are on the

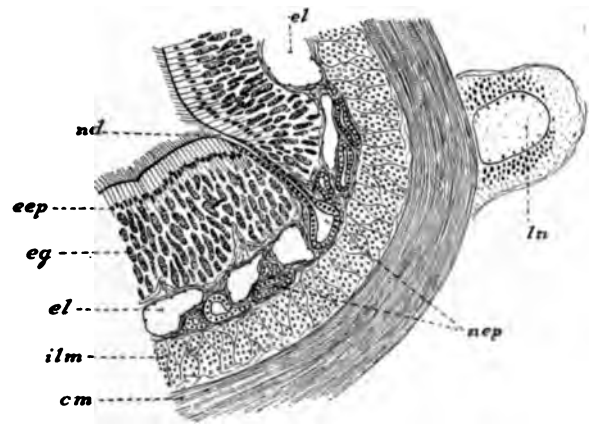

FIG. 2. *Tæniosoma cingulatum.* Portion of transverse section of body through middle of esophageal region, showing efferent nephridial duct opening into lumen of esophagus. Reference letters as in Fig. 1. × 130.

ventro-lateral borders of the esophagus and in the vicinity of the lateral nerves (Figs. 1, 2, 3).

During the movements of the body the cavity of the esophagus is continually changing in outline, and the epithelial lining is thrown up into numerous temporary longitudinal ridges. Such ridges may form either in the vicinity of the nephridial openings or between them. In the former case the nephridial duct is greatly elongated and very slender and opens on the side or

summit of the ridge, whereas if the opening is between two such ridges the duct is necessarily short and has a correspondingly thicker epithelial lining. The length of the nephridial duct must thus vary in accord with different degrees of extension of the esophageal walls and the accompanying changes in the height of the longitudinal ridges.

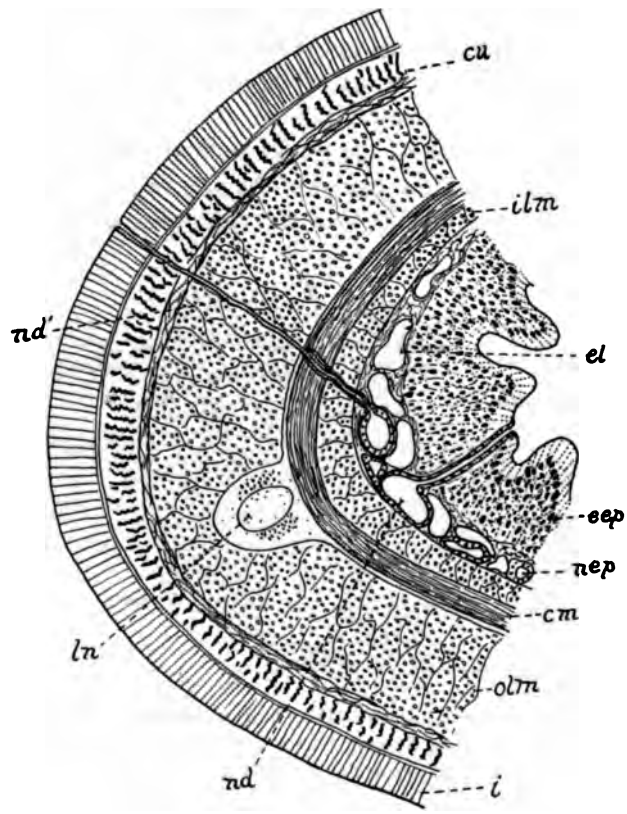

FIG. 3. *Tæniosoma cingulatum.* Diagram of portion of transverse section of body through esophageal region, showing an efferent nephridial duct (*nd*) opening into lumen of esophagus and another (*nd'*) leading to dorso-lateral surface of body. Reference letters as in Fig. 1. × 50.

The nephridial system appears to be continuous throughout its whole length. From the larger canals originate both the ducts opening into the esophagus and those opening externally (Fig. 3). The excretory fluids may be discharged in either

direction, but probably pass mainly into the esophagus. Such a disposition of these waste products need not occasion any surprise when one considers how important a part the esophagus plays in respiration. I have often observed a species of the genus (*Tæniosoma delineatum*) with its mouth and esophagus widely dilated, while the rhythmic contractions of the body walls caused a comparatively large volume of water to be taken into the esophagus and then discharged through the mouth. In this manner the walls of the esophagus are constantly bathed with a regularly changing supply of water. Excretory products poured into the lumen of this portion of the alimentary canal would thus be quickly discharged from the body through the mouth. When, on the other hand, the esophagus is filled with food — a large annelid, for example — the pressure on the esophageal walls would very likely be sufficient to close up the nephridial ducts opening into this cavity, and in such a case the efferent ducts opening externally would be brought into service, and the excretory products be discharged through them.

The path from the nephridial canals to the esophageal lumen is much more direct than that through the thick muscular walls to the exterior of the body. Hence, with an abundant means of constantly flushing out the esophagus in the act of respiration, the animal economy is as well served by the discharge internally as by a direct flow to the exterior.

Only in such forms as have large mouths and use the esophagus as a respiratory organ would this be the case, however.

Although the two species mentioned are the only nemerteans in which this peculiar condition of the nephridial ducts is known to exist, yet in one other species of the same genus (*Tæniosoma indicum*) Punnett[1] has found fine cords of cells resembling delicate ducts, compressed so as to obscure the lumen, passing into the epithelium of the esophagus, although no actual openings were found. Neither did he find any ducts opening externally. And in some of the other species, belonging to different genera where careful study has as yet failed to recognize the efferent ducts, perhaps

[1] Punnett, R. C., "Fauna and Geography of the Maldive and Laccadive Archipelagoes," Vol. 1, part 1, p. 105, 1901.

a search for the esophageal nephridial ducts would meet with success.

Obviously the excretory apparatus of the nemerteans has undergone numerous and striking modifications from the primitive planarian type so well represented in the Bdellonemertea and in numerous species of each of the other orders.

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BIOLOGICAL BULLETIN

ON THE INFLUENCE OF ELECTROLYTES UPON THE RATE OF RHYTHMIC MUSCULAR CONTRACTIONS (PRELIMINARY COMMUNICATION).

T. BRAILSFORD ROBERTSON.

[*From the Rudolph Spreckles Physiological Laboratory of the University of California.*]

1. In a previous paper[1] I have suggested that the rate of beat of a rhythmically contracting tissue, such as the heart, may be connected with the velocities of the ions in the tissue-fluids, or in the medium in which the heart is suspended, by the following formula:

$$t = a \cdot \frac{u}{v(u+v)} + b,$$

where t is the interval between the beginning of one beat and the beginning of the next, u is the average velocity of the kations in the medium, v that of the anions in the medium and a and b are constants for any given heart.

2. The assumptions involved in the deduction of this formula are: (1) That the ions diffuse into the tissue in numbers proportional to their migration-velocities in water; (2) that they give rise to a reversible chemical change in the tissue, a change consisting in the displacement of ions already present in the tissue in ion-proteid compounds by those which have newly entered, and that this reaction obeys Guldberg and Waage's mass-law and (3) that the ions thus displaced in each successive segment of tissue give rise to the contraction.

[1] *Pflüger's Arch. f. d. ges. Physiol.*, Bd. 110, p. 610.

3. It occurred to me that, provided the above-mentioned assumptions were correct, the efficacy of a saline medium in maintaining the heart-beat at a normal rate should depend upon two factors, namely, the reversibility of the chemical changes inaugurated by it, and the value of $u/[v(u + v)]$ for the solution. Any solution bringing about only reversible changes (that is, for example, not containing heavy metals) and having the same value of $u/[v(u + v)]$, as Ringer's solution, should be equally efficacious in maintaining the heart-beat. Loeb has introduced the conception of Physiologically Balanced Salt Solutions.[1] It may be that as far as rhythmically contracting tissues are concerned the value of $u/[v(u + v)]$ may afford us a clue to the theoretical composition of such solutions.

4. I made up a Ringer solution with the following composition: $N/10$ NaCl + $N/250$ KCl + $N/200$ $CaCl_2$, which proved to be a good heart solution. The NaCl was made free from potassium by volatilizing the KCl out of Kahlbaum's C.P.NaCl, KCl having a lower boiling-point than NaCl. The average value of u for such a solution can be found by multiplying the velocity of each kation by its equivalent molecular concentration, and dividing the sum of these products by the sum of the concentrations. The value of $u/[v(u + v)]$ for this Ringer solution, calculated from the values for the ionic velocities given in Landolt-Börnstein,[2] is 621.2×10^{-5}. A solution of $LiNO_3$, NH_4NO_3 and $NaNO_3$ having approximately the same value for $u/[v(u + v)]$ is one made up as follows: $.135N$ $LiNO_3$ + $.03N$ NH_4NO_3 + $.01N$ $NaNO_3$—the value of $u/[v(u + v)]$ (calculated from the table of ionic velocities quoted above) is 628×10^{-5}. This forms an excellent heart solution; twenty-three variations of this solution were tested, keeping the concentration of the $LiNO_3$ constant, and varying that of the NH_4NO_3 and $NaNO_3$. In solutions 1–5 the $LiNO_3$ and $NaNO_3$ were kept at $.135N$ and $.015N$ respectively, and the NH_4NO_3 varied from $.03N$ to $045N$, increasing in concentration in each solution from 1 to 5 by $.005N$. In solutions 6–9 the concentrations of the $LiNO_3$ and NH_4NO_3 were kept at $.135N$

[1] *American Journal of Physiology*, Vol. III. (1900), p. 431, "Studies in General Biology," Decennial Publications of the University of Chicago, Vol. II., p. 590.

[2] *Physikalische-Chemische Tabellen*, 1905.

and .03N respectively, and that of the $NaNO_3$ was raised from 0.15N to .035N, differing by .005N in each successive solution.

Solution 10 was the calculated solution. In solutions 11–15 the concentrations of the $LiNO_3$ and $NaNO_3$ were kept at .135N and .015N respectively, while the concentration of the NH_4NO_3 was decreased from .02N in 11 to .000N in 15 by steps of .005N. In solutions 16–19 the concentrations of the $LiNO_3$ and $NaNO_3$ were kept at .135N and .015N respectively, while that of the NH_4NO_3 was raised from .031N in 16 to .034N in 19 by steps of .001N. In solutions 20–23 the concentrations of the $LiNO_3$ and NH_4NO_3 were kept at .135N and .03N respectively, and the concentration of the $NaNO_3$ raised from .016N to .019N in steps of .001N. Out of all the twenty-three solutions thus tried the only ones which approximated to the Ringer as sustainers of the heart-beat were the calculated solution (10) and solution 1 (.135N $LiNO_3$ + .03N NH_4NO_3 + .015N $NaNO_3$) in which $u/[v(u + v)] = 629.5$ — and these are the two solutions which approximate most closely to Ringer in the value of $u/[v(u + v)]$. Thus solution 11 (.135N $LiNO_3$ + .02N NH_4NO_3 + .015N $NaNO_3$) was much less efficacious than the calculated solution. On estimating the value of $u/[v(u + v)]$ for this solution the reason becomes clear, for its value is 615.1, a value departing comparatively widely from that for Ringer or for solution 10. The other solutions in which the NH_4NO_3 concentration was still further lowered (11–15) were still more deleterious. Raising the concentration of the $NaNO_3$ above that in solution 1 was also deleterious (solutions 6–9), and it will be found that in these solutions the value of $u/[v(u + v)]$ rises above 630, as might be expected; raising the concentration of the NH_4NO_3 (solutions 1–5) was still more deleterious.

In solution 10, to quote one experiment, a heart washed in NaCl and beating initially at a rate of 10 in 15 seconds, continued beating for 65 minutes; in 1, a heart beating initially at the same rate and treated in the same way as the above, and at the same time, beat for from 40–60 minutes; while a third heart, in Ringer, and beating initially at the same rate as the other two, beat for 90 minutes.

5. Thinking that perhaps the concentrations of these solutions were somewhat too high to be altogether favorable, I made up a series of six solutions with the concentration of $LiNO_3$ in each .1N — one, solution 24, was calculated so as to give a value of $u/[v(u + v)]$ approximating to that in Ringer — its composition was .1$N$$LiNO_3$ + .02$N$$NH_4NO_3$ + .015$N$$NaNO_3$, and the value of $u/[v(u + v)]$ was 626.7. The remainder were solutions containing the same concentrations of $LiNO_3$ and $NaNO_3$, but varying in NH_4NO_3 concentration from .02N (24) to .025N (29). The most favorable solution was 24, the others departing more widely in the value of $u/[v(u + v)]$ from that for Ringer.

This new calculated solution formed a really excellent sustainer of the heart-beat, and by the addition of a trace (1 c.c. $N/40$ to 100 c.c. solution) of Na_2CO_3, to give a favorable alkalinity, a solution was obtained which was in every way as favorable a medium for the heart-beat as the Ringer to which the same amount of Na_2CO_3 had been added. Frogs' hearts beat in these solutions (24 + Na_2CO_3 and Ringer + Na_2CO_3) for over two hours.

6. Thus the facts are in favor of the hypothesis, and we see that neither K, Ca or Cl ions are specific and essential for the heart-beat, nor, probably, Na ions — all that is necessary is to obtain a properly balanced solution containing no ions (such as heavy metals) which make irreversible compounds with proteids, and having the most favorable value of $u/[v(u + v)]$. Such a solution could be made up from any two or three salts whose values of $u/[v(u + v)]$ differed sufficiently widely in opposite directions from the value for Ringer. It is not inconceivable, of course, that a favorable heart solution may ultimately be found which contains a heavy metal — but one would not expect to find such a solution having the same value of $u/[v(u + v)]$ as Ringer, since the constants a and b (in the equation which I have quoted) would probably be altered by the heavy metal.

These experiments will be continued.

7. In the course of an investigation on the influence of temperature upon the rate of the heart-beat in *Ceriodaphnia* the results of which have appeared in the BIOLOGICAL BULLETIN,[1] I found that the rate of the heart-beat in *Ceriodaphnia* is practically constant

[1] Vol. X., p. 242 (1906).

at 21° C., provided the different individuals are taken from the same vessel, treated in the same way, and not previously exposed to any extreme temperatures. With these precautions individuals differing widely in development will be found to have practically the same rate of heart-beat at 21°. *Ceriodaphnia* can therefore be used as homogeneous material for the investigation of the influence of electrolytes upon the rate of the heat-beat — that is, the constants a and b in the equation

$$t = a \cdot \frac{u}{v(u+v)} + b$$

are practically the same for all the individuals, and the influence of each solution can be tested upon a fresh individual. The number of beats was registered upon a revolving drum by tapping a key in a circuit containing a signal-magnet, and the time was taken with a stop-watch.

8. The following are the experimental results so far obtained, the "time of one beat" meaning the interval between the beginning of one beat and the beginning of the next. The values of $u/[v(u+v)]$ are calculated from the table of ionic-velocities to which I have previously referred. In every case the organisms were first washed in tap-water, then placed in about 50 cc. of the solution for 10 minutes and then placed, with a drop of solution, on a glass plate kept accurately at 21° by means of a stream of water running underneath — in 10 minutes the rate of beat was noted.

Solution	$u/[v(u+v)]$	Time of one beat.	Value of a when $b = 1.7232$.
$N/10$ $LiNO_3$	567.7×10^{-5}	.3 sec.	356.4
$N/10$ NaCl	611.6×10^{-5}	.150 "	306.6
Ringer	621.2×10^{-5}	.183 "	30 .9
100 c.c. Ringer + 2 cc. $N/10$ KCl	624.1×10^{-5}	.185 "	305.75
$N/10$ $MgCl_2$	631.4×10^{-5}	.2 "	304.6
40 c.c. $N/10$ NaCl + 10 c.c. $N/10$ KCl	645.9×10^{-5}	.238 "	303.7
30 c.c. $N/10$ NaCl + 20 c.c. $N/10$ KCl	677.8×10^{-5}	.353 "	306.6
30 c.c. $N/10$ $LiNO_3$ + 20c.c. $N/10$ NH_4CNS	725.1×10^{-5}	.375 "	289.4
$N/10$ NH_4CNS	937.6×10^{-5}	.5 "	237.1

Thus we see that the value of a is constant for values of $u/[v(u+v)]$ between and including 611.6×10^{-5} and 677.8×10^{-5}. Above and below those values it is variable.

9. It is evident that upper and lower limiting values of $u/[v(u+v)]$ exist between which sustained beats are possible.

The existence of the lower limit is evident from a consideration of the equation

$$t = \frac{au}{v'(u + v)} + b,$$

for since b has a negative sign, when

$$\frac{u}{v'(u + v)} = -\frac{b}{a}$$

then the time of one beat is zero, and for still lower values of $u/[v'(u + v)]$ the time of one beat would be negative, which is, of course, impossible. The equation

$$\frac{u}{v'(u + v)} = -\frac{b}{a},$$

therefore gives us a theoretical lower limit, in this case 562×10^{-5}. Before this, however, as the experiment with $LiNO_3$ shows, the beats begin to fuse — for the beats take a certain time to traverse the heart, and this interval may be greater than that between the beginnings of two beats. The upper limit is possibly the expression of the fact that ions must reach the minimum number necessary to displace those in combination with the proteids (the "threshold-number") before they have time to diffuse away through the tissue. Before this point, however, irregularities appear, as in the last two experiments in the table above. The beats soon stop in these extreme solutions. Thus in KCl or in 20 c.c. $N/10$ NaCl + 30 c.c. $N/10$ KCl the beats are only sustained for about 5 minutes at 21° — in the latter solution the value of $u/[v'(u + v)]$ is 706.9, and we notice that it is just about at this value of $u/[v'(u + v)]$ that irregularities begin to appear in the value of the constant a. Hence the limits for *Ceriodaphnia* are probably 570×10^{-5} and 700×10^{-5}. Any solution in which $u/[v'(u + v)]$ lies between these values, and which does not contain any substance forming irreversible proteid compounds, should sustain the heart-beat in this organism — solutions having values of $u/[v'(u + v)]$ falling outside these limits could not sustain the heart-beat.

10. By mixing a solution having a value of $u/[v'(u + v)]$ below the lower limit for the rhythmically contracting tissue in question

with one having a value of $u/[v(u + v')]$ above the upper limit, a solution could evidently be obtained which would sustain the heart-beat. In this way many salt-antagonisms could be obtained. Thus I have found that the heart of *Ceriodaphnia* will not beat in $N/10$ KCl or in 20 c.c. $N/10$ NaCl + 30 c.c. $N/10$ KCl, but will beat in 30 c.c. $N/10$ NaCl + 20 c.c. $N/10$ KCl, because the two former solutions have values of $u/[v(u + v)]$ lying above the upper limit for this rhythmically beating tissue, while the last-mentioned solution has a value of $u/[v(u + v)]$ lying between the upper and lower limits. If we were considering the question as one of toxicity, we should say that NaCl *antagonized* KCl. The possible reason for the antagonism is now clear.

11. The values of the upper and lower limits for rhythmical contractions in a tissue will obviously depend upon the constants a and b for that tissue in the equation

$$t = a \cdot \frac{u}{v(u + v')} + b.$$

These constants will be different in different tissues, and need not be the same for different parts of the same tissue. Thus, in the case of *Gonionemus*, it is only necessary to suppose that the limits for the center lie very close on either side of the value of $u/[v(u + v)]$ for NaCl, while the limits for the margin are somewhat farther apart, to obtain a possible explanation of the fact that the center will beat in NaCl solution but not in NaCl solution to which KCl or $CaCl_2$ have been added, while the *margin* will continue to beat in such solutions.[1] In this way many of the apparently specific effects of certain ions upon rhythmically contracting tissues may possibly be reduced to a general explanation.

12. Experiments will be continued with a view to obtaining further evidence as to the existence of upper and lower limits in the value of $u/[v(u + v)]$, above and below which rhythmic contractions are impossible, and also further experiments upon the degree to which the crustacean heart obeys the formula

$$t = a \cdot \frac{u}{v(u + v')} + b.$$

[1] Loeb, *American Journal of Physiology*, Vol. III. (1900), p. 383.

THE EYES OF THE BLIND VERTEBRATES OF NORTH AMERICA.

VII. The Eyes of Amphisbæna punctata (Bell), A Blind Lizard From Cuba.[1]

FERNANDUS PAYNE.

Amphisbæna punctata is a blind legless lizard which burrows in the ground. It is common in Cuba to which it is restricted. How deep it burrows, I do not know, but it is often turned out by the plow. The specimens obtained ranged from 103 to 245 mm. in length. The head is short, hard and pointed, and the tip of the upper jaw projects slightly beyond the tip of the lower jaw. In shape, arrangement of the dermal plates, and in the color of the ventral surface of the body it closely resembles an earthworm. The dorsal surface is flesh-color with small brown spots. The tail is short and flattened dorsoventrally. In a specimen 245 mm. in length, there were 225 annuli on the dorsal side, 202 on the ventral and 15 on the tail. In this specimen the tail was one thirteenth and the head one thirty-fifth the length of the body.

Methods. — The lizards were put alive into formalin. They were afterwards changed to alcohol. For decalcification, the heads were placed in five per cent. nitric acid from twenty to thirty days. A shorter period did not give satisfactory results. Some of the heads were imbedded in paraffin and others in paraffin and celloidin. In using the latter method I imbedded the head in celloidin in the usual manner and hardened in chloroform. From chloroform I transferred the block to soft paraffin for twenty-four hours and thence to hard paraffin for twenty-four hours, after which I imbedded the block in paraffin.

[1] Contribution from the Zoölogical laboratory of Indiana University, No. 77. The material used in the preparation of this paper was incidentally collected during several expeditions to Cuba. The prime object of the expeditions was to collect life history material of the Cuban blind fishes, *Lucifuga* and *Stygicola*. They were undertaken with a grant of $1,000 from the Carnegie Institution.

The best results were obtained from those imbedded in paraffin and celloidin. Several methods of staining were used; iron hæmatoxylin with eosin as a counterstain gave the best results. The more modern methods of treating the retina with silver could not be applied for lack of fresh specimens. On account of the extreme toughness of the cuticle it was impossible to get complete series of sections. For comparison I have examined the eye of *Anolis carolinensis*.

General Account of the Eye.

The eye of *Amphisbæna* appears indistinctly as a small black spot beneath the ocular plate (Fig. 1). In a specimen 225 mm. in length, the eye is 352 μ beneath the surface, 420 μ in width and 360 μ in depth. The conjunctival sac is 116 μ in diameter. The conjunctiva is very thin over the cornea, but measures 4 μ in thickness over the anterior part of the sac.

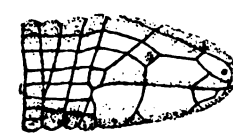

Fig. 1.

The dermis and epidermis have the same structure over the eye as over the regions near by. This corresponds with what Eigenmann ("The Eyes of *Rhineura floridana*," 1902) found in *Rhineura*, although the eye of *Rhineura* is a much more degenerate organ than the eye of *Amphisbæna*. To what extent the eye has degenerated from a more elaborate structure I am unable to say. Few organs are stationary and this one is probably still in process of reduction. I have been unable to obtain the young, and there is no means of finding out from the adult whether the eye is degenerating at present or not. In each specimen examined the eyes appeared in about the same state of degeneration.

The eye measures 1,224 μ in circumference and the pupil 104 μ in diameter. The uveal part of the iris on each side of the pupil measures 250 μ. The pupil and iris occupy 49.3 per cent. or very nearly half of the entire circumference.

Harder's gland is very much larger than the eye. In a cross-section through the central portion of the eye, the antero-posterior diameter is approximately three times and the medio-lateral diameter four times the medio-lateral diameter of the eye. It is

divided into two distinct lobes, the anterior being much smaller than the posterior. The gland completely surrounds the eye except over the anterior face. Its secretion is poured into the conjunctival sac and from thence into the mouth cavity. The large size of the gland in *Typhlops* led Duvernoy to the conclusion that its function was not connected with the eye. As its secretion, in *Amphisbæna*, is poured into the conjunctival sac and thence into the mouth cavity, its function must have been, primarily at least, connected with the eye.

No eye muscles are present in *Amphisbæna*.

The eye is directed outward and forward and makes an angle of about 60° with a line drawn tangent to the dermal plate which covers it.

Whether the eye is still used as a sense organ, I cannot say, but since the parts are so well developed and the eye is not buried very deeply beneath the surface, I am inclined to believe that it is at least, susceptible to light.

The Sclera. — The sclera (*scl.*, Fig. 6) has apparently undergone no degeneration whatever. It compares favorably with that of *Anolis*. In fact, there is but little difference in its structure in the two eyes. At the proximal part of the eye, the sclera measures 12 μ in thickness, while at the same place in *Anolis* it measures 15 μ. It is continuous over the front of the lens as the cornea, which together with the thin wall of the conjunctival sac at this place measures 7 μ. Scleral cartilages extend from about the middle of the eye back almost to the optic nerve. On each side of the sclera and forming a part of it, are thin irregular layers of pigment in patches.

The Choroid. — If the blood vessels in the choroid still persist, the preparations do not show them. All that can be seen is a number of densely pigmented cells, around and between which are filaments of connective tissue (*chr.*, Fig. 6). At the entrance of the optic nerve, this layer measures 8 μ in thickness, but gradually becomes less forward and vanishes entirely a short distance back of the enlarged end of the pigment layer. The pecten, present in *Anolis*, is not apparent in *Amphisbæna*.

The Lens. — The lens has retained its natural shape and position (*lens*, Fig. 6). It is almost spherical and measures 80 μ in

diameter. In most of the sections an outer layer of cells extends around the anterior surface of the lens. The interior in nearly every case stained as a structureless mass, but in a few sections it appeared to be made up of large irregularly shaped cells with small nuclei. If any fibrous cells still persist, they did not show. No capsule is present.

The Vitreous Body. — The vitreous body (*vit. cav.*, Fig. 6) occupies the greater part of the eye-ball and has certainly undergone but little change. The aqueous cavity has entirely disappeared.

The Iris. — Only the uveal part of the iris remains. It is continuous with the pigment epithelium of the retina and has the same structure. In the thickest part it measures 68 μ. The cells are similar to those of the pigment layer, except that their radial diameter is much greater. The ciliary processes are no longer present.

The Optic Nerve. — The optic nerve can be traced from the eye, through and along the side of Harder's gland. While the nerve could be traced no further on account of an incomplete series of sections, there is no doubt that the connection with the brain still exists. The nerve fibers enter the eye in a compact mass, pass through the layers of the retina until they reach the nerve fiber layer, where they spread out and connect with the nerve cells of the ganglionic layer in the usual manner.

The Retina.

While the retina has undergone considerable change, all the layers are still present (Fig. 3). It measures 78 μ in thickness. In *Anolis* about half way between the anterior and posterior parts of the eye it is 179 μ in thickness. If the macula lutea is still present, the preparations do not show it.

The Pigment Layer. — The pigment layer (1, Fig. 6) which bounds the retina externally, consists of a single stratum of rectangular cells separated by a small amount of clear intercellular substance. These cells have large oval nuclei free from pigment, almost transparent and with small nucleoli. At the back portion of the eye where the pigment layer measures 8 μ, the transverse diameter of the cells is greater than the radial diameter, but

toward the anterior portion where the layer becomes thicker, the radial diameter becomes much the greater. The greatest thickness of this layer is near the lens, where it measures 68 μ. The outer surface of the pigment cells — that which lies next to the choroid — is smooth and slightly convex. The inner surface, on the other hand, is very irregular. The cells at this place are very densely laden with pigment and prolonged into filamentous processes which extend between and amongst the cones. In fact, the cones may be said to be imbedded in the pigment cells. This layer differs but little from that of *Anolis*, except at the anterior part of the eye where it becomes much thicker.

The Cones. — No rods are present. The cones (2, Fig. 3) consist of an upper and a basal portion. The basal part is elliptical in shape and stains uniformly throughout, while the outer portion is longer and somewhat triangular in shape, with the smaller side of the triangle resting on the inner elliptical part. This layer measures 10 μ in depth while the same layer in *Anolis* measures 13 μ.

The Outer Nuclear Layer. — This layer is made up of a single stratum of nuclei with small dark nucleoli (3, Fig. 3). Some of these nuclei are almost spherical, while others are oval in shape. They are connected with the cones by broad processes which stain darkly. These processes may be very short, in which case the cone comes in close proximity to the nucleus; or they may be drawn out into filaments as long as or longer than the nuclei themselves. From the inner part of the nuclei extend processes which broaden toward the base and send numerous ramifications into the inner stratum of the outer reticular layer. There is a striking difference here between this eye and the normal one. The processes from the base of the nuclei pass straight through the outer reticular layer while in my sections of the normal eye, they pass through at an angle of about 45° (3, Fig. 4).

The Outer Reticular Layer. — The outer reticular layer (4, Fig. 3), is penetrated by the processes from the nuclei of the outer nuclear layer and by a few Müllerian fibers. If processes from horizontal cells are present they were not brought out by the method of staining which was used. Again, there is but little

difference in the thickness of this layer in the two eyes, as it measures 6 μ in *Amphisbæna* and 7 μ in *Anolis*.

The Inner Nuclear Layer. — The inner nuclear layer is a compact mass of somewhat irregular spherical nuclei and is 24 μ in thickness (6, Fig. 3). The corresponding layer in *Anolis* is 59 μ. Spongioblast and bipolar cells cannot be differentiated from each other. All the nuclei appear to be very much alike, except the nucleated enlargements of the fibers of Müller, which have no definite shape and which stain very densely. However, some nuclei, more especially those of the inner stratum, stain a very deep black color, and show no structure whatever. Parts of certain other nuclei stain densely, while the rest retains its original identity. Some of the nuclei have from four to six nucleoli. In *Anolis* two other kinds of nuclei appear. A few flattened horizontal nuclei can be seen near the middle of the layer and in the inner stratum are a number of large spherical nuclei. Penetrating this layer are many fibers of Müller. Each fiber as it passes through is characterized by a nucleated enlargement.

The Inner Reticular Layer. — The inner reticular layer measures 20 μ in thickness as against 45 μ in *Anolis* (8, Figs. 3 and 4). The method of staining brought out no definite structures. The fibers of Müller pass through it as fine vertical filaments. Occasionally there is a nucleus from the nuclear layer or from the ganglionic layer which lies imbedded in the edge of this layer.

The Ganglionic Layer. — The ganglionic layer (9, Fig. 3) consists of a single layer of nuclei 6 μ in diameter, with now and then another nucleus above or below the single layer. From the outer side of these nuclei fibers which run out and penetrate the inner reticular layer, can be traced for a short distance. On the opposite side are also fibers which continue as fibers of the nerve fiber layer. In *Anolis* this layer measures 23 μ and is made up of loosely connected nuclei, some of which are large and spherical, others are smaller and irregular, while still others stain very densely.

The Nerve Fiber Layer. — The nerve fiber layer is 6 μ in depth while in *Anolis* it is 26 μ.

The Fibers of Müller. — The Müllerian fibers can be traced from the membrana limitans interna to the outer nuclear layer.

They commence at the inner surface of the retina by a broad conical foot which extends into the ganglionic layer. Through the inner reticular layer the fibers pass as fine filaments, but in the inner nuclear layer each fiber is characterized by an irregularly shaped nucleus, which stains densely and shows no structure. The membrana limitans externa is not visible. These fibers differ but little from those in *Anolis*, except that those in *Anolis* can be traced to the membrana limitans externa, which is plainly visible.

SUMMARY.

1. The eye muscles have entirely disappeared.
2. Only the uveal parts of the iris remain.
3. The lens has retained its shape and position, but its structure has been greatly changed. No capsule is present.
4. Harder's gland is many times larger than the eye and pours its secretion into the conjunctival cavity and thence into the mouth.
5. The sclera, scleral cartilages, cornea, vitrous body and pigment epithelium have undergone but little change unless it be in the reduction in size.
6. The cuticle passes over the eye unchanged.
7. The aqueous cavity is no longer present.
8. All the layers of the retina are still present. As shown in Fig. 6, the great reductions in the depth of the layers, in comparison with those of *Anolis*, has taken place in the nerve fiber, ganglion cell, inner reticular and inner nuclear layers.
9. If the eye has been reduced from an eye of the average size, all parts have certainly undergone considerable change, and this change has been approximately equal among the several parts.
10. The retina does not show such a profound change as either the iris, muscles or lens. However, it has been greatly changed, as it extends only 50.7 per cent. of the distance around the eye.
11. The eye of *Amphisbæna* bears out the statement made by Eigenmann ("Eyes of the Blind Vertebrates of North America," I.) that the more active parts of the eye are the ones to degenerate first. They are the parts which have been most affected.

ACKNOWLEDGMENTS.

I took up the work on this lizard at the suggestion of Dr. C. H. Eigenmann and it is under his direction that the work has been carried forward. To him I am indebted for many helpful suggestions and for my specimens. I also wish to express my thanks to Mr. Leonard Haseman, of Lake City, Florida, for sending me specimens of *Anolis*.

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Explanation of Plate I.

1, pigment layer; 2, cones; 3, outer nuclear layer; 4, outer reticular layer; 6, inner nuclear layer; 8, inner reticular layer; 9, ganglion cell layer; 10, fiber layer; *lens*, lens; *scl.*, sclerotic; *chr.*, choroid; *cor.*, cornea; *scl.c.*, scleral cartilage; *n.op.*, optic nerve; *vit.cav.*, vitreous cavity; *con.cav.*, conjunctival cavity; *C.*, outer covering of the eye; *M.*, Müllerian fiber; *L.*, membrana limitans externa.

Figs. 1, 2 and 5 were drawn from sections. One-twelfth objective and one-inch eye-piece.

Figs. 4 and 6 are diagrammatic.

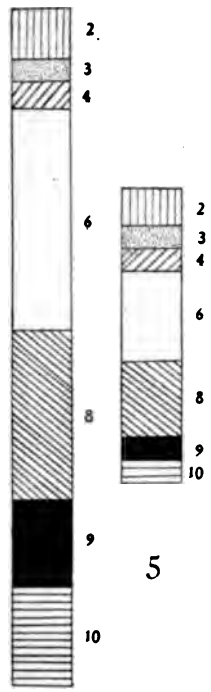
Fig. 1. Side view of the head.

Fig. 2. Diagram of the eye showing the parts in their relation and the distance of the eye beneath the surface.

Fig. 3. Horizontal section of the retina of *Amphisbæna punctata*, showing the different layers.

Fig. 4. Horizontal section of the retina of *Anolis*.

Fig. 5. Diagram showing the comparative measurements of the retina in the two eyes.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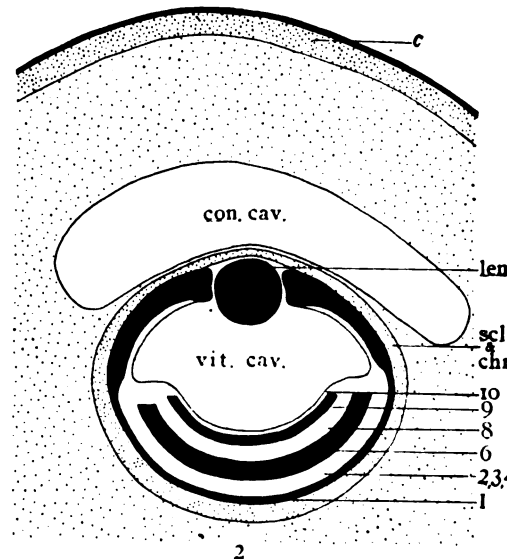
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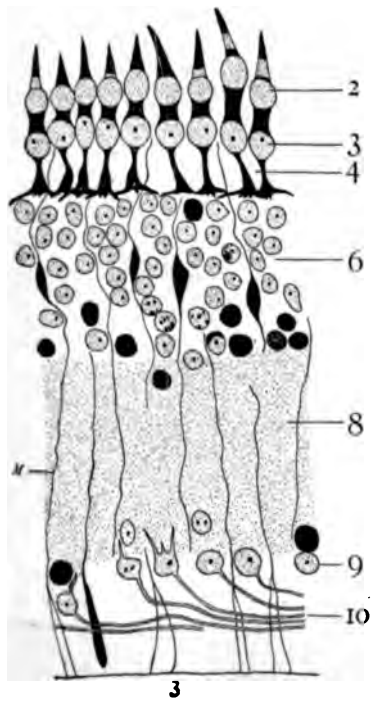
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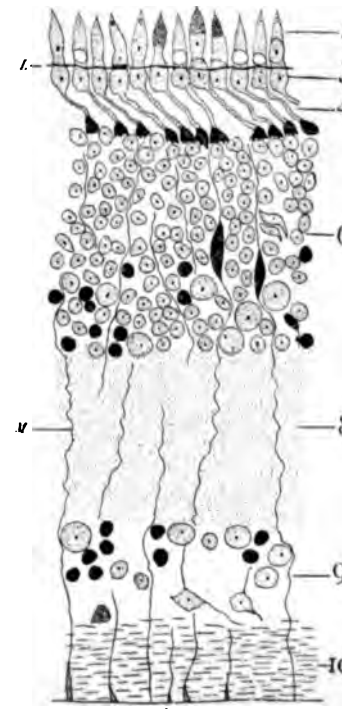
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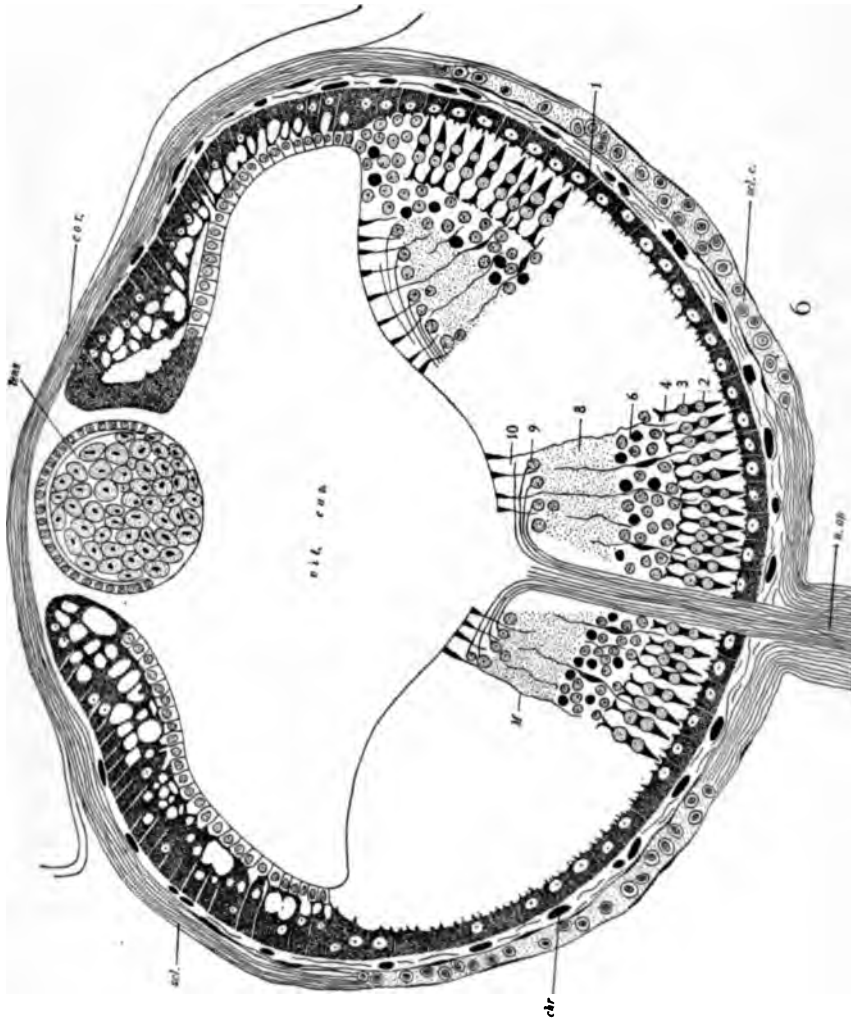
EXPLANATION OF PLATE II.

FIG. 6. Horizontal section of the eye showing the different parts. The retina is diagrammatic.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EXPERIMENTS WITH FROG'S EGGS.

T. H. MORGAN.

The following experiments were carried out in part during the spring of 1905, and in part during the present year. As the headings of the different sections indicate I have brought together the results of experiments of very different kinds, but since they all bear on the same questions it seemed preferable to put the results together in one paper, rather than to scatter them through several. The following topics are discussed: (1) The Development of the Frog's Egg out of Water, (2) The Increase in the Size of the Egg during the Segmentation Stages, (3) Obliteration of the Blastocœl by means of a Centrifugal Force and the Effect on the Subsequent Development, (4) Removal of the Roof of the Blastocœl, (5) Effects of Cold on the Early Development, (6) The Early Development of the Lithium Larvæ of the Frog, (7) Effects of Lithium Chlorid and Sodium Chlorid Acting Together, (8) Effects of Lithium Chlorid and Magnesium Chlorid Acting Together, (9) The Chemical Versus the Osmotic Effects of Salt Solutions.

The Development of the Frog's Egg Out of Water.

In order to determine whether the segmentation cavity of the frog's egg is simply a water-filled space, left by the cells of the blastula as they separate, or whether the cavity is formed by the active secretion of the surrounding cells, I placed eggs on pieces of filter paper and allowed them to develop out of water. On the first assumption, the blastocœl is filled with water, that, percolating *between* the cells, passes into the interior. The enlargement of the normal egg during the cleavage period shows that water is really absorbed by the egg, but whether this water simply fills the enlarging segmentation cavity, or whether it enters the cells could only be determined by keeping the eggs out of water during the early development.

In the first experiment the eggs were placed on pieces of wet filter paper after their outer membranes had been removed. The

... into water. Under these
... and young tadpoles ap-
... ed enough water from the
... ent. In later experiments,
... branes, were placed on dry
... on each small piece. The
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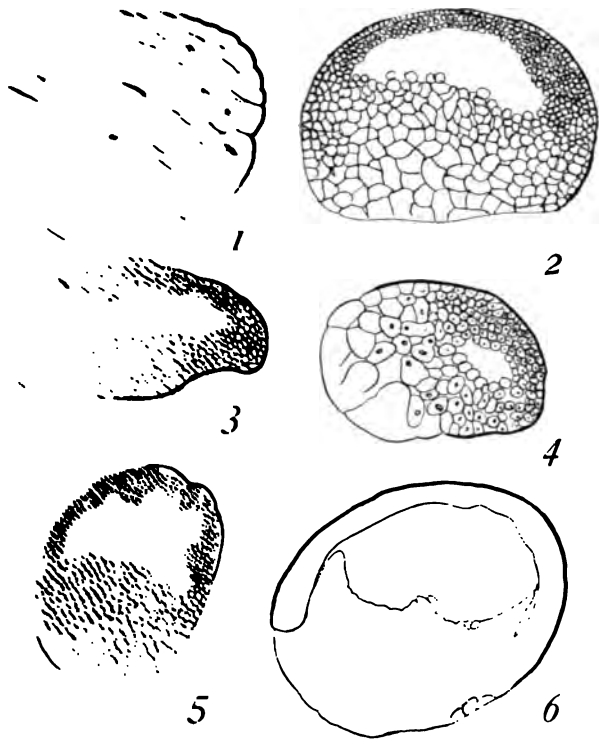
... paper were then put into glasses
... drying. In the course of a few
... on the side in contact with the
... inued under these conditions, and



... Eggs developing out of water

... noticeably those where too much water had been
... abnormal embryos developed. In the majority
... however, the egg died, or the development was retarded

in the late cleavage stages, the amount of desiccation determining the result. Despite the drying of the egg with its accompanying flattening, the segmentation cavity appeared in it, as sections showed, and while it generally failed to reach the proportions characteristic of normal development (except when the paper was too wet), yet that it should develop at all under the adverse conditions of the experiment, demonstrates that its contents are produced, in part at least, as a secretion of the surrounding cells. A few sections of these eggs are reproduced in Figs. 1–12.

A section through an egg that had been placed on a dry piece of filter paper at the two-cell stage, and killed some hours later, is shown in Fig. 1. A large segmentation cavity is present in the interior although the egg is flattened below from pressure.

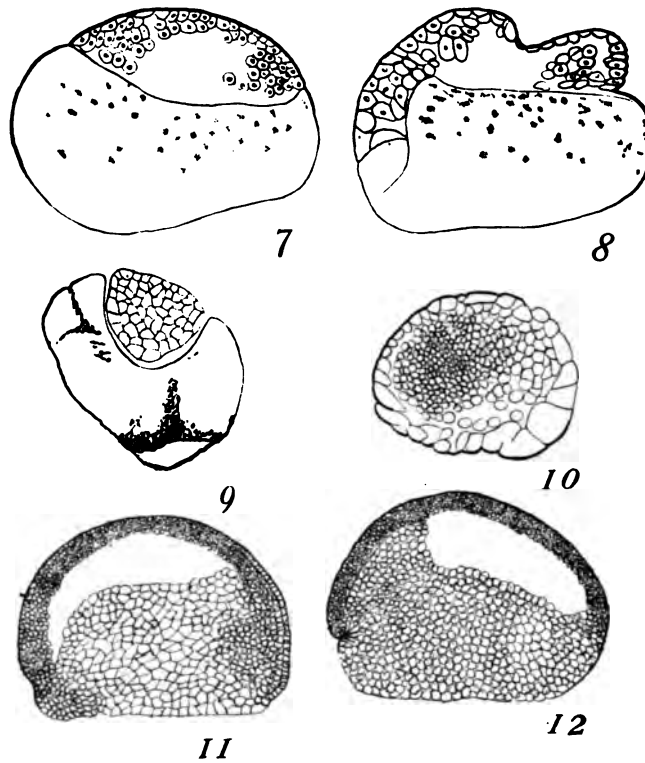
A later stage is shown in Fig. 2. The egg was attached by its white pole. The blastocœl is large and not very different from that of a normal egg. A still later stage of an egg, that was still further compressed (on one side in this case), is shown in Fig. 3. The segmentation cavity is smaller than in the normal blastula of this age. The reduction is probably connected with the great flattening of the egg. Similarly in the next figure, Fig. 4, the segmentation cavity is reduced in size. In the next two figures, Figs. 5 and 6, the segmentation cavity is larger, and as large, in fact, as that of the normal egg. These eggs were but little compressed, and, therefore, developed more nearly in the normal manner.

The next two figures, Figs. 7 and 8, show a different type of development. The yolk has been so much injured by the drying, that it has failed to segment, yet it is filled with nuclei each surrounded by pigment. Both of these eggs were fastened by the lower hemisphere. The tops of the eggs were less injured, and had divided into small cells, that arch over a large segmentation cavity.

The next egg is very different, Fig. 9. Here, too, the yolk has been killed, but there is present a solid mass of cells imbedded in the yolk. The egg seems to have been attached to one side of the black hemisphere, and the small cells in the interior are due to the shifting of the interior of the egg. The next figure, Fig. 10,

shows a somewhat similar condition, where, however, the lighter material of the top of the egg has sunken into the interior. This was due to the eggs being placed on the paper in an inverted position while in the two-cell stage. The figures show that the smallest cells are in the interior of the egg, and not on the surface of the black hemisphere.

In order to meet the possible objection that the egg may absorb water from the small, slightly damp, piece of paper, another series



FIGS. 7–12. Eggs developing out of water.

of experiments was carried out, in which the eggs, deprived of their outer coats, were kept on pieces of glass. For several minutes the eggs were left on the glass exposed to the air, in order that the water sticking to their outer coats, or to the glass, might dry off. They were then placed in the moist chamber. An egg that had reached a late blastula stage under these conditions is

shown in Fig. 11, and another at the beginning of gastrulation in Fig. 12. Both show a large segmentation cavity. The results may be summed up as follows: If the egg in the two- or four cell-stage is taken from the water and kept from drying, it will develop, and the segmentation cavity will be formed, which, although sometimes smaller than the normal, if the egg is very dry, yet its presence under these adverse conditions shows that it must be due to a secretion poured out by the blastula cells, and that it is not due directly to the passage of the water from the outside into the egg.

I have found that the eggs of *Fundulus heteroclitus* will also develop out of water if simply placed on a glass plate in a moist atmosphere. The eggs of the starfish will pass through several segmentation stages under similar conditions, but so little water remains on the glass that it evaporates quickly and the eggs are so delicate that they cannot withstand the drying. The blastula becomes flattened and is nearly solid, but on being placed again in sea water it quickly rounds up, and the segmentation cavity appears. The normal segmentation cavity in the starfish is very large and is early formed. Its absence in the eggs out of water may be due to the flattening of the egg, but possibly its absence is due to the necessity of the cells to absorb water in order that it may be formed. Those who have studied the segmentation of the sea urchin egg in water under compression, as when it is placed between compressing plates, have observed the absence of the segmentation cavity, even when the segmenting egg has become two-layered. In the frog also, as we have seen, the compression may be responsible for the suppression of the full development of the blastocœl, but since the compression is not carried so far in the latter case, the formation of the cavity is not suppressed, and the fact that it forms at all under these adverse conditions goes to show that its origin is due to the activity of the surrounding cells.

The Increase in the Size of the Egg During the Segmentation Stages and the Increase of the Blastocœl.

I measured some eggs of *Rana sylvestris* at different stages of development in order to see whether the increase in size of the

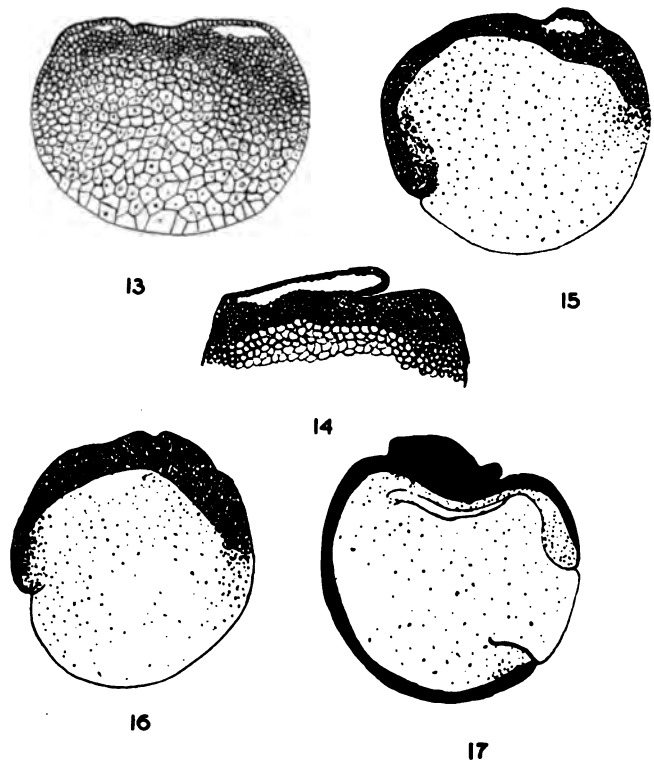
egg during the segmentation stages and pregastrula stages is equal to or greater than the space of the blastocœl. On account of the variation in size of different eggs it would have been better to measure the same egg at different times. I shall give some measurements of several eggs of the same bunch but not the same eggs. In one case the unsegmented eggs measured 5.1, 5.2, 5.3 and 5.4. The average eggs seem about 5.3. At the beginning of gastrulation the eggs measured 5.6 and 5.7. When the dorsal lip was widely horseshoe-shaped the eggs measured 5.6 and 5.8; and when the gastrula lips were nearly close 5.8 and 5.9. In another case the unsegmented eggs measured 5.2, 5.3, 5.4 and at the beginning of gastrulation 5.5, 5.6, 5.7. In another case the unsegmented eggs measured 5.2, 5.3, 5.4, and at the beginning of gastrulation 5.4, 5.5, 5.8.

If we assume that the unsegmented egg measures 5.3 and that the egg about to gastrulate measures 5.6, the latter will have gained about two ninths of the volume of the former, or roughly one fourth. An approximate estimate of the size of the blastocœl when fully formed, as compared with the blastula in which it is contained, shows that the blastocœl is about one eighth the entire volume. Hence, while the egg has gained one fourth in volume, only one eighth of its increase is due to the segmentation cavity; *i. e.*, only one half of the increase in size can be accounted for by the segmentation cavity alone, and the other half must have been due to the absorption of water by the cells of the blastula. Thus we must conclude that the egg is absorbing water during the segmentation stages, and that at the same time it is giving up to the blastocœl an amount of fluid that is approximately half of the amount absorbed. If the egg is placed under conditions where it can not absorb water, it gives up, nevertheless, nearly the normal amount to the blastocœl.

Obliteration of the Blastocœl by Means of a Centrifugal Force and the Effect on Subsequent Development.

The eggs of *Rana sylvestris* in a late blastula stage were put into tubes of water and revolved on a small centrifuge at the rate of 1,600 revolutions per minute for ten minutes. At the end of

this time the black hemisphere of the egg was much flattened, and sections show, Fig. 13, that the fluid of the blastocœl has been completely driven out of the egg. The figure shows that not only has the top of the egg been flattened, but the small cells at the sides of the blastocœl have moved over across the top of the yolk, so that a thick layer of small cells lies as a flat plate on top of the larger yolk cells. Here and there a small crack or space is present along the region of contact of the large and the small cells. Not infrequently the top of the egg is thrown over as a fold, as shown in Fig. 14. This is due no doubt to the fact that as an arch it occupies more space than when flattened down on the egg. In the preserved eggs an artefact often appears beneath the outer layer of cells.



FIGS. 13–17. Eggs of *Rana sylvestris* after having been rotated at 1,600 revolutions per minute. 13, immediately after rotating; 14, top of another egg at same time; 15–17, later stages when gastrulation has begun.

These eggs gastrulated and produced embryos. Sections through several of the intermediate stages show that, after the removal from the machine, the top of the egg did not regain its former roundness, Fig. 15, but remained flattened. In a few cases a small space was found near the top of the egg beneath the ectoderm that may represent a part of the blastocœl, Fig. 16. A later stage is shown in Fig. 17. The blastopore is closing, the archenteron is present, but no indications of the blastocœl can be found. A lump of ectoderm lies near the anterior end of the embryo. It is an almost constant feature of these embryos, and owes its origin to the injury of the roof of the blastocœl. Its location does not necessarily mean that the normal embryo extends to the top of the egg, because extensive movements of the cap of small cells must take place during the time of gastrulation. Nevertheless it is true that this lump of cells originated near the top of the egg, and had been carried downwards towards the anterior end of the embryo. In fact the material of which it is composed may represent the ectoderm of the anterior end that had been carried upwards by the action of the centrifugal force.

The results show that the blastocœl is not essential for the formation of the frog embryo, since the process of gastrulation may take place in its absence. This does not mean that the blastocœl may not be made use of in ordinary development; in fact it is made use of, since the yolk mass is thrown into it, but the result does mean that the blastocœl is not essential for development. Two methods of interpreting the blastocœl have been followed by embryologists. The commonly accepted method is to "explain" it by assigning to it a purpose. Its purpose is to make a space into which the yolk cells can be thrown at the time of gastrulation. It would seem from this point of view that the blastocœl is not only a useful, but also an essential organ in development. The results show, however, that this is not the case.

The other method of interpretation is that of the school of developmental mechanics which has tried to account for the formation of the blastocœl as the results of some such mechanical process as infiltration, and have assigned it to the function of producing an osmotic pressure on the walls of the blastula. Rhumbler

has tried to account for the gastrulation process as the outcome of the accumulation of certain waste products in the blastocœl, but since the development of the gastrula may take place when no blastocœl is present, this explanation of the mechanics of gastrulation does not appeal to me as a probable one.

Removal of the Roof of the Blastocœl.

It has been suggested by Rhumbler that the process of gastrulation may be due to the accumulation of waste products, carbon dioxide for instance, in the blastocœl fluid. The presence of such a substance would bring about changes in the surface tension on one side of the yolk cells, which, by causing them to change shape, is imagined to bring about the inturning of the cells. It seemed to me that this view might be tested by emptying the blastocœl of its fluid before gastrulation had occurred. If the inturning still took place the result would show that the process need not be connected with the blastocœl fluid, or with substances that have become dissolved in it.

There was also another question that I wished to examine by means of the same experiment. The formation of the large blastocœl space takes place at the time when the embryo-forming materials, that come from the upper hemisphere, are moving outwards and downwards at the sides of the blastocœl, and the question arises whether this movement is connected with the development of the blastocœl space. Finally there is still a third question involved, namely, whether the movement of the material is due to the downward pressure of the cells themselves of the roof of the blastocœl.

In the first set of experiments the roof of the blastocœl was opened, and injured by plunging a needle into it. Despite the operation the process of gastrulation still took place in most cases, and a normal embryo developed. In consequence of the operation, as sections show, a large part of the fluid of the blastocœl is set free, although a small part of it may remain. When the operation is carried out at an early stage the cavity may develop later and suffice to bring about the gastrulation, if it were really due to this factor. In order to meet this possible objection, I operated on two other sets of eggs, one at the time when the

blastocœl had reached its highest point of development, and the other at the time when the dorsal lip of the blastopore had just appeared on the surface. Sections showed that the blastocœl was emptied in large part, yet in both cases gastrulation took place. There is, however, to be noticed a distinct retardation in the time of gastrulating between the normal and the injured eggs. The hole made in the roof closes almost at once, but a lump of cells generally indicates, throughout the gastrulation process, the place of injury. Owing to the closure of the top, the pressure relations of the cells will be again largely reëstablished, but the delay in the time of gastrulation indicates that the injury has to some extent interfered with the processes involved in the act of gastrulating.

The results show that Rhumbler's hypothesis is probably incorrect at least so far as the blastocœl is concerned. If however he should shift his position and assume that it is the accumulation of waste substances in the interior of the egg itself, *i. e.*, in the middle of the yolk-cells, that causes the invagination, the results are not fatal to his view. I attempted therefore to test Rhumbler's hypothesis in another way. If the amount of carbon dioxide outside the egg can be made equal to that in the interior, the process of gastrulation should not occur, if Rhumbler's view is correct. I placed frog's eggs in the late blastula stages in a bottle containing some water. By means of a tube running beneath the water I forced air that I had held in my lungs for a few seconds through this water. The air above the water was also displaced through an outlet. The communications with the outside were then closed. It seemed probable that there would be as much, and probably a great deal more carbon dioxide outside the eggs than inside, yet during the following six to twelve hours the gastrulation took place in the normal manner. This result also is not favorable to Rhumbler's interpretation. Other observations and experiments have led me to think that the process of gastrulation cannot be explained by such mechanical processes as surface tension, and I have tried to show elsewhere [1] that the change in shape of the cells that leads to the invagination is due to a process of active contraction of the cells, which

[1] *Roux's Archiv*, XIX., 1905.

in turn is the outcome of the pressure relations of the cells on each other.

Effects of Cold on the Early Development.

In a preceding paper[1] I have described some abnormal embryos of the frog that were produced as the result of cold. The early segmentation stages of these eggs were not preserved, but I can now make good this deficiency by means of a series of young stages kept under similar condition (1° to 2° C.), that have been put up for me by Dr. N. M. Stevens.

The most obvious effect of the cold is of course to delay the development. The secondary effects involve (1) greater injury to the yolk cells than to those of the upper hemisphere, (2) effects on the formation of the segmentation cavity, and (3) in some cases the retention of the small cells at the upper hemisphere. How far this latter change may be directly connected with the injury to the lower cells, so that they fail to draw inwards, is a point difficult to determine, but if the cold is responsible for the failure of these cells to draw inwards, the retention of the small cells at the upper pole may directly result.

The details of the development of these eggs, left on the ice for eleven days, are as follows (the eggs were put on the ice April 9, at 10 a. m.):

April 9, 1 p. m. Some eggs still in the four-cell stage, others going into eight cells.

April 9, 6 p. m. Eight-cell stage. Small segmentation cavity present.

April 10, 9 a. m. Some eggs going into the twelve-cell stage. The segmentation cavity is well developed (Fig. 18).

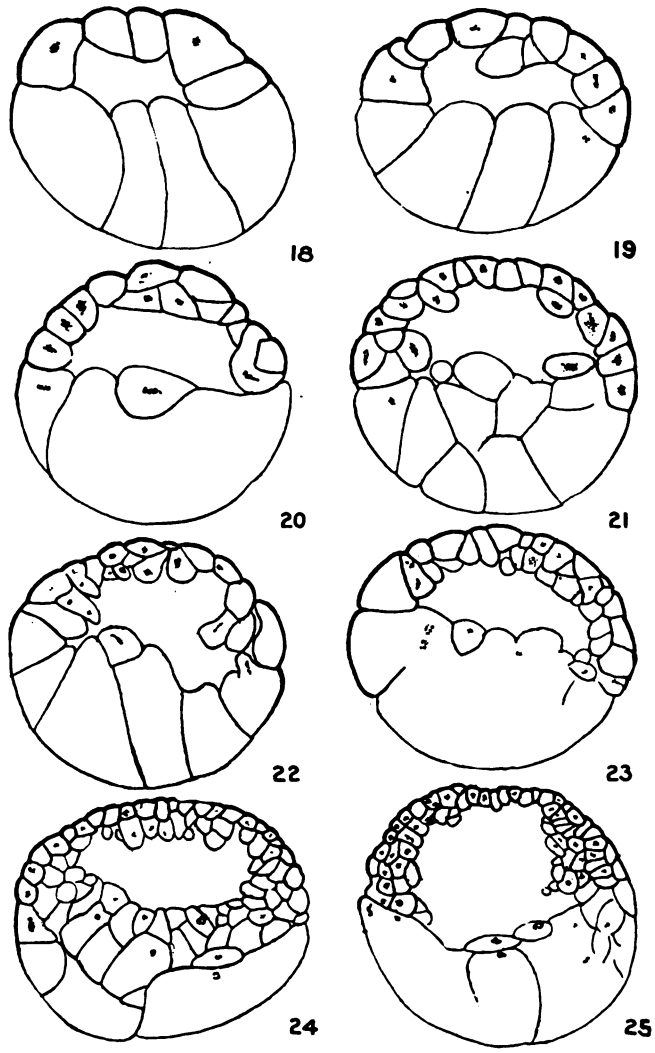
April 10, 5 p. m. Sixteen-cell stage. The segmentation cavity extends far down into the yolk.

April 11, 9 a. m. Another cell division has taken place. A large segmention cavity has developed (Fig. 19).

April 11, 8 p. m. Immigration of cells at the top of the egg taking place. Segmentation cavity better developed on one side than on the other.

[1] *Roux's Archiv*, XIX., 1905.

April 12, 9 a. m. Further division has occurred. Black cells are noticeably confined to the upper third of the egg, *i. e.*, there is not the normal downgrowth (Fig. 20).



FIGS. 18–25. Segmentation stages of egg kept on ice. *Rana palustris.*

April 12, 6 p. m. Condition same as last. Segmentation cavity large.

April 13, 9 a. m. Same condition; possibly another division has occurred.

April 13, 6 p. m. Some of the eggs have divided further. Large blastocœl (Fig. 21).

April 14, 9 a. m. Same as last.

April 14, 5:15 p. m. A large and irregular segmentation cavity, lying more on one side, is seen in sections (Fig. 22).

April 15, 9 a. m. The surface of the egg shows white flecks, and the cells have divided further. Large blastocœl present.

April 16, 2 p. m. Same. Cells irregular. Segmentation cavity large.

April 18, 9 a. m. Same. Large segmentation cavity. No downgrowth of small cells. Yolk irregularly divided (Fig. 23).

April 20, 9 a. m. Divided further. Top of egg brown instead of black. Large and irregular segmentation cavity (Fig. 24).

April 22, 9 a. m. In the surface view the outlines of the upper dark cells are very irregular. Large segmentation cavity surrounded by irregular cells (Fig. 25).

Compared with the normal set,[1] put up at the same time, the retardation of this cold series is apparent. After eleven days these eggs on the ice did not develop further than those at room temperature in twenty-four hours. Not only is there a delay, but the development has become quite abnormal, especially during the later stages. The sections show that the cells have in general an irregular outline, and are not compacted together as in the normal embryo. There is no downward migration of the material of the top of the egg, hence the abnormal forms of embryos described in my former paper. Great differences and abnormalities are found in the formation of the segmentation cavity, and this same difference was noticeable in the older stages. These older stages have been described in my earlier papers.

The Early Development of Lithium Larvæ of the Frog.

In a previous paper[2] I have described some of the effects of lithium solutions on the development of the frog's egg. The

[1] The control normal set are those described in my forthcoming paper on "The Origin of the Embryo-Forming Materials in the Frog's Embryo," 1906.

[2] *Roux's Archiv*, XVI., 1903.

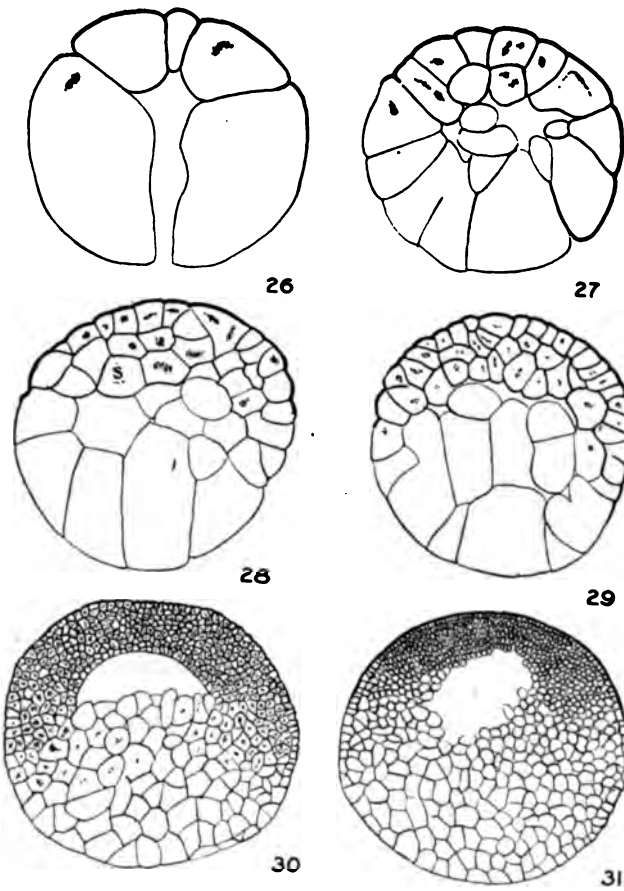
most peculiar and interesting type of these larvæ is that in which the embryo is formed in the interior of the egg instead of on the surface. In other cases the material of the top of the egg accumulates as a solid black cap upon which the anterior end of the neural plate sometimes appears. Embryos also appear in which the dark cells extend to different distances over the yolk.

The material that I used to study these embryos lacked the earlier stages, and while the observations on the living eggs left little doubt that the results were due to the failure of the upper cells to move downwards over the yolk, yet the actual details were not known. Since the question of the origin of the embryo-forming material is involved in my interpretation of the results it was desirable to obtain these missing stages. Dr. N. M. Stevens has kindly put up for me the necessary material for studying these missing stages of *Rana palustris*, the same species that I formerly used.

The eggs in the two-cell stage were put into a 0.5 per cent. solution of lithium chlorid and preserved at intervals of two hours. After the first two hours the eggs were in the eight-cell stage. A section through one of these is represented in Fig. 26. It differs little from a normal egg.[1] The segmentation cavity is somewhat smaller than that of the normal egg at this time. Two hours later the egg had reached the condition shown in Fig. 27. The inpulling of the cells of the upper hemisphere has begun. The segmentation cavity is noticeably small. Two hours later, Fig. 28, the divisions have gone further, and the upper cells now form almost a solid cap around the upper pole. A very small segmentation cavity, *S*, is present. Two hours later, Fig. 29, the smaller cells at the top still remain in place. A flattened segmentation cavity is present, but much reduced as compared with that of the normal egg. During the next four hours the changes are not marked. The small cells remain at the top and the segmentation cavity remains small. Two hours after this, *i. e.*, six hours after the last stage figured, the eggs are in the condition represented in Fig. 30. The roof of the segmentation cavity is very thick and the small cells still remain in the upper hemisphere. Four hours later, Fig. 31, the material at the top of the

[1] In preserving the lower blastomeres became separated in the egg.

egg has concentrated in a cap. It represents all of the ectoderm of the embryo. This brings the development to the stage at which my former series begins. If, after this stage is reached, the ectodermal material at the top of the egg turns into the interior, instead of growing down over the surface, as in the normal egg, an invaginated embryo is formed.



FIGS. 26–31. Segmentation stages of egg in lithium chloride. *Rana palustris.*

If it fails to turn in, it remains as a solid cap at the top, and if not too condensed the anterior end of the neural plate develops.

In the lithium types there is rather a sharp line of demarcation between ectoderm, mesoderm and endoderm. The three layers

appear to be more or less stratified in the order just given. This is best seen in the inverted type, in which the ectoderm turns in at the top. The mesoderm forms a sheet of cells wrapped around the neural plate, and the endoderm is drawn upwards as a sheet of cells over the mesoderm. (See Fig. I, Pl. XXIV., in my paper of 1903).[1] The endodermal layer, that is drawn upwards, appears to be that which is normally turned in around the lips of the circular blastopores. In addition to this there is always formed a tube opening on the surface that is lined by yolk cells. The opening of this tube lies near the equator of the egg and forms a crescent-shaped mouth there. The cells that line this tube correspond, I think, to those that make the anterior end of the normal archenteron. This part of the archenteron of the normal embryo appears to develop by the drawing apart of the yolk cells.

The mesoderm of the lithium larvæ is made up of the cells that lie just beneath the ectodermal cap. If we may draw any conclusion from the condition of the mesoderm in the lithium forms in regard to the origin of the first formed mesoderm of the normal embryo, we would conclude that it does not come from those cells of the 32- to 64-cell stages that are drawn beneath the surface at the top of the egg, but from cells lower down at about the level of the upper portions of the lower four blastomeres of the eight-cell stage. There may be, however, not a little latitude in respect to the potency of many of these cells, and their location is probably also a factor in their differentiation.

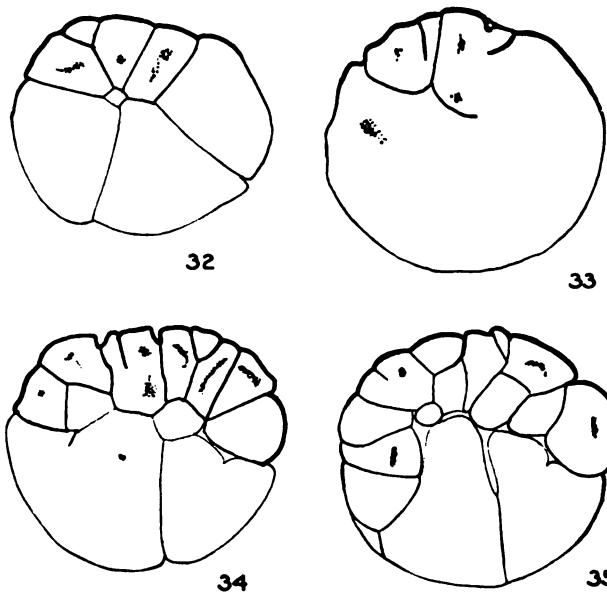
Effects of Lithium Chlorid and Sodium Chlorid Acting Together.

The effect of lithium seems to depend in large part upon chemical, rather than upon osmotic, effects. Other salts also act chemically in somewhat the same way as lithium, but to a less degree. I have tried the effects of these salts acting together and also separately on the eggs of *Rana palustris* and of *Bufo lentiginosus*. The material for the former species I owe to Dr. Stevens.

A solution of LiCl 0.5 and NaCl 0.6 per cent. gave the results shown in Figs. 32 and 33. The cleavage was not only greatly

[1] *Roux's Archiv*, XVI.

retarded but more irregular than normal, and came to an end before many divisions had been carried out. The details of the results were as follows: —



FIGS. 32–33. Segmentation of egg in lithium chloride and sodium chloride. *Rana palustris.*

FIGS. 34–35. Segmentation of egg in lithium chloride and magnesium chloride. *Rana palustris.*

The eggs in the two-cell stage were put into the double solution. After two hours a normal looking two-cell stage was present. After another two hours the cells had divided, and were in the same condition two hours later, Fig. 32. The egg shows little or no segmentation cavity. Other eggs have an abnormally small segmentation cavity. After two hours more, the upper cells have again divided, and the upper ends of the lower cells also. After two hours more, the eggs appear to be in the same condition, and this is also true for the next six hours. Sections of these eggs show a tendency for the blastomeres to run together (Fig. 33), and each contains several nuclei. After a further interval of eight hours, the cell-walls have largely disappeared on the surface.

The results show very clearly that the double solution has acted more injuriously on the egg than the lithium chlorid alone. The results appear, from a comparison with sugar solutions, not to be entirely due to the increase in osmotic pressure, but to the greater effect of the combined action of the sodium and the lithium.

Effects of Lithium Chlorid and Magnesium Chlorid Acting Together.

Eggs in the two-cell stage were put into a solution containing LiCl 0.5 + $MgCl_2$ 0.6 per cent. After two hours the eggs were still in the two-cell stage. Two hours later the eggs were in the 16-cell stage, some of them having divided with great irregularity. Sections show a small segmentation cavity present. Two hours later, Figs. 34 and 35, one or two further divisions have taken place. As shown in these figures the egg is nearly solid, and in other eggs there is no trace of the segmentation cavity. Another division has taken place after two hours more, and another division after another two hours. At this time the sections show the interior cell-walls running together to produce polynuclear cells. Two hours later the egg is as before, or has divided again, and after another four hours further division has occurred, but in this and the following stages (after eight hours), the cells especially the yolk cells are becoming polynuclear and division is apparently near its end without having produced a large number of cells. It is noticeable that the yolk cells have been more affected than the smaller cells of the upper hemisphere. The results are similar on the whole to those with LiCl + NaCl, but less marked; the younger stages being more regular. The segmentation cavity appears to have been practically obliterated.

The Chemical versus the Osmotic Effects of the Salt Solutions.

In order to determine, if possible, how far the action of the salt solutions is due to chemical effects and how far to osmotic effects, the following experiments were carried out with the eggs of *Rana sylvatica*. Eggs in the two-cell stage were put into solutions

of graded strengths. The amount of salt is given in percentages although this is not quite accurate, since, in the first place, one per cent., for example, is one gram added to 100 cc. (and not to 99 cc.) of water; and in the second place a certain further decrease in strength is caused by the water in the jelly around the eggs.

It is not an easy matter to determine definitely where normal development ceases, for, not only is the development often stopped at different stages in the same solution, but there is also a considerable overlapping in the different solutions. I have arbitrarily selected as the upper limit, the strength of solution in which, although the cleavage may continue for a time, gastrulation does not occur.

For LiCl alone it was found that gastrulation may take place and a normal embryo appear in a 0.5 per cent. solution. In a 0.55 per cent. solution the blastopore is large, its closure is delayed, and even prevented. In a 0.6 per cent. solution the blastopore appears, but generally no embryo develops; while the upper limit seems to be about 0.65 per cent. where practically all of the eggs fail to pass beyond the late segmentation stages. For NaCl the upper limit is above 2.0; but owing to an accident the upper limit was not accurately determined.

In a solution of LiCl 0.4 per cent. + NaCl 0.3 per cent. only the late segmentation stages appeared. Thus both solutions, being lower than the maximum for each, produce an injurious effect. In another double solution of LiCl 0.5 per cent. + NaCl 0.5 per cent. a few nearly normal embryos developed, but in a solution of LiCl 0.5 per cent. + NaCl 1.0 per cent. only the late segmentation stages developed, or rather a cap of black cells appeared at the top of the egg (surrounded in some cases by a band of gray cells, as in lithium larvæ that I formerly described). In a solution of LiCl 0.5 per cent. + NaCl 1.5 per cent. only late segmentation stages developed. This then may be taken as the limit.

A solution of cane sugar must be quite strong in order to prevent the development of the embryo. Even a 6.0 per cent. solution gives normal embryos. An 8 per cent. delays the closure of the blastopore, and a 10 per cent., while not preventing the appearance of the blastopore, does prevent its closure,

and no embryo develops. In a 12 per cent. solution the egg reaches only the late cleavage stages.

In a solution of LiCl 0.4 per cent. + sugar 5.0 per cent. only about the sixty-fourth-cell stage is reached. Thus together the two substances produce an effect that neither alone affects. In another case, LiCl 0.5 per cent. + sugar 1.0 per cent. delayed the closure of the blastopore; a solution of LiCl 0.5 per cent. + sugar 2.0 per cent. also delayed, but did not actually prevent the closure of the blastopore; while a solution of LiCl 0.5 per cent. + sugar 4.0 per cent. stopped the development of most of the eggs in the late cleavage; although in one case at least the outline of the neural plate appeared. The results show that the sugar reinforces the effects (or a part of it at least) of the lithium. It remains now to see whether the results can be explained as due to osmotic pressure alone or whether a chemical effect also occurs.

Concentration.		*Ionization.*	*Osmotic Pressure at 18° C. in Atmospheres.*
Per Cent.	Mols Per Liter.	Per Cent.	
Cane Sugar.			
1	0.029	0	0.698
2	0.058		1.396
4	0.117		2.792
6	0.175		4.188
10	0.292		6.980
12	0.351		8.376
Lithium chlorid.			
0.4	0.094	82.3	3.843
0.5	0.118	82.3	4.804
0.55	0.129	82.3	5.284
0.6	0.141	79.9	5.690
0.65	0.153	79.9	6.161
Sodium chlorid.			
0.3	0.051	87.4	2.15
0.5	0.085	85.2	3.55
1.0	0.171	81.8	6.96
1.5	0.256	79 1	10.28
2	0.342	77.7	13.61
3	0.513	73.7	19.95
4	0.684	71.6	26.28
Lithium chlorid + Sodium chlorid.			
0.4	0.3		5.99
0.5	0.5		8.35
0.5	1.0		11.76
Lithium chlorid + Sugar.			
0.4	5.0		7.33
0.5	1.0		5.50
0.5	4.0		7.50

In the accompanying table, kindly prepared by Dr. H. W. Berg, the osmotic pressures of most of the solutions given in the preceding statement are given. It will be seen for the upper limit of LiCl, namely, 0.65 per cent., the osmotic pressure is 6.161. In comparison the results with NaCl are very different. The upper limit is above 2 per cent. The osmotic pressure for 2 per cent. is 13.61, which is more than double the strength of effective LiCl. The comparison shows that the effects of the lithium salt are not due to osmotic pressure alone.

In a double solution containing LiCl 0.5 + NaCl 0.5 nearly normal embryos appeared, and the osmotic pressure in this case is 8.33, which is much greater than that for the lithium alone. It took, in fact, a solution containing LiCl 0.5 + NaCl 1.0 to prevent gastrulation. The osmotic pressure in this case is 11.76, a pressure far greater than that for the effective limit of LiCl alone.

The upper limit for cane sugar was found to be about 12 per cent., which corresponds to an osmotic pressure of 8.376. This is much higher than for the lithium chlorid alone.

For solutions of LiCl 0.4 + sugar 5.0 per cent., only the 64-cell stage was reached. The osmotic pressure is 7.33, which is again much higher than for lithium chloride alone, but less than that for the sugar alone. It would seem, therefore, that the two together must have a higher pressure than for the one producing its effects at the lower limit, but less than for the other that produces its effects at a higher pressure. Similar conclusions might be drawn from the double solution containing LiCl 0.5 + sugar 4.0 per cent. The results show that there is a double effect produced by salt solutions, a chemical and an osmotic. How each effect is produced we do not know at present. Loeb has shown, however, that living substances behave very differently towards the amount of water absorbed according to what chemical element, sodium, calcium, or lithium, for example, that they have taken up. Since the development of the embryo is associated with the amount of water absorbed it might appear that in this way the chemical action is similar to the results produced by means of osmotic pressures which also effect the amount of water. That the egg contains enough water in itself for normal development is shown by my

experiments of causing it to develop out of water, but this does not show that if water is removed beyond a certain point or the capacity to absorb water is changed the development might not be delayed. In fact if the egg is dried too much it fails to develop. However this may be, the point of special interest brought out by these experiments is that an effect may be produced by a double solution in which the total osmotic pressure is lower than that required to produce the effect by one of the substances alone, but higher than that sufficient to produce the result by the other alone. It seems probable that the effect is a double one ; in part chemical, in part osmotic.

OBSERVATIONS ON THE REACTIONS OF CRYPTOBRANCHUS AND NECTURUS TO LIGHT AND HEAT.

A. M. REESE.

During most of the time for the past four years the writer has had one or more specimens of the giant salamander (*C. alleghenicnsis*) in captivity in the laboratory, and the tendency of these animals to seek the darker parts of the tanks in which they were confined, or to crawl under any sufficiently large object that might be present, led to the experiments which are summarized in the present paper.

The experiments with *Necturus* were performed on five individuals of average size, though most of the light experiments were carried on upon one individual.

Four large specimens of *Cryptobranchus* were used for both sets of experiments, the heat experiments being nearly all performed after the completion of the light experiments.

In the light experiments three sources of illumination were employed: A sixteen candle-power incandescent electric lamp, so shaded that the light could be thrown on any given part of the animal without illuminating the rest of the tank; the direct rays of the sun, reflected from a small mirror; and an ordinary arc, projection lantern, which was set up at the side of the tank, so that a narrow beam of light could be reflected from a mirror into the water.

In the color experiments red and blue globes were used with the incandescent lights, and with the other two methods of illumination plates of red and blue glass were introduced into the white rays from the sun or from the electric arc.

It was found, by the use of the spectroscope, that the red plate gave an almost pure red, while the red bulb gave, besides the red, some yellow and green rays. The blue plate gave the entire spectrum except the dark green, yellow and yellowish red; the blue bulb gave the entire spectrum, which apparently differed

from that of the ordinary white bulb only in intensity. Owing to this impurity in the colors, especially the blue, the observations with the red and blue lights cannot be given much weight; some of the observations will, however, be briefly given below.

It was found that both with *Cryptobranchus* and *Necturus* the responses to light were much more marked for the first ten or a dozen stimulations than for succeeding stimulations, so that it was necessary, on account of this loss of sensitiveness, to make only a comparatively short series of experiments at any one time. Ordinarily these animals will lie for many minutes or, possibly, even hours without the slightest motion, so that their reaction to stimuli at these times is too evident to doubt; but occasionally, especially at night, they become restless, and it becomes necessary to postpone experimentation. In each case where no reaction was obtained within two and one half minutes the reaction was recorded as "none." Since the animals under observation were at all times covered with several inches of water it seemed unnecessary to use any form of heat screen, as this depth of water would absorb all heat rays from the artificial lights if not from the solar illumination.

Reactions of Cryptobranchus to Light.

The effect of a ray of white light when thrown on different parts of the body was first tried. It was found that all parts of the body are sensitive to white light, but that the tail is by far the most sensitive region. While enough experiments were performed to show that the middle regions were more or less sensitive to white rays, it was the head and tail that were chiefly studied in their reactions to light stimulation.

When a ray of white light was thrown upon the tail the response was, in very many cases, immediate, and consisted in a quick forward movement of the animal until the tail was removed from the illumined area. In almost no cases among many trials was the response delayed for as much as a quarter of a minute, the average time for a response being about three seconds or less. The extreme sensitiveness of the tail of this animal to light stimulation is quite remarkable, and the response is, in all cases, exactly the same.

In about half of the experiments where light was thrown upon the head of *Cryptobranchus* no response was obtained even at the end of two and a half minutes or longer, and in no case was a response obtained in less than nine seconds: however, sufficient undoubted responses were obtained to justify the statement that the head of the animal is sensitive to white light. The response in this case, when it came at all, was invariably shown by the animal's backing away from the light.

The responses were about as sudden and as strong when the incandescent light was used as when the much stronger illuminations were employed; this may have been due to the fact that when the rays from the sun or the arc light were used the tank was partially illumined by diffused light, while in the other case there was no light except the small circle that came from the shaded incandescent bulb.

That *Cryptobranchus* is sensitive to white light of even weak intensity seems proven by the fact that the animals seek the darker parts of their tank in ordinary diffused light.

The results obtained with the red and blue lights were not so definite as those with the white light, and, as has been previously stated, may have but little value. With the red plate which gave, with the arc light, a strong, pure red illumination, no responses at all were obtained, either from head or tail stimulation. The incandescent light with the ruby globe, while not so strong a light, produced, in most cases, decided responses; this was possibly due to the fact that the light was not nearly so pure a red as that given by the plate. The responses were of the same character as with the white light, the difference being one of rapidity.

In no case was the response to a stimulus instantaneous, as with the white light, but in several instances reaction took place, when the tail was stimulated, within two seconds. As with the white light, the head was much less sensitive than the tail.

The reactions to blue light were of the same character as with the other forms of illumination, but were more rapid than with the red light. The apparently greater sensitiveness to blue than to red light may have been due simply to the greater purity of the latter color.

Reactions of Necturus to Light.

The same kinds of experiments were tried with *Necturus* as with *Cryptobranchus*. All parts of the body seemed sensitive to white light, but in this case the head was more sensitive than the tail, the withdrawal from the circle of illumination being instantaneous, in many cases.

The effect of illumination from below was also tried with *Necturus*, the animals being placed in a glass aquarium which was shaded from above, while a beam of sunlight was thrown on the ventral regions of the body. All parts of the ventral surface were sensitive to this form of stimulation, but the head was, in this case, much less sensitive than the tail. The responses as a whole were neither so quick nor so strong as when the light fell upon the dorsal regions of the body.

A beam of red light produced by the same red plate that was used in the experiments upon *Cryptobranchus* produced no reactions that were definite enough to be of value. The red, incandescent bulb, on the other hand, caused fairly strong reactions, those from the head and tail being of about the same suddenness.

The reactions to blue light, whether produced by the plate or the incandescent bulb, were much more decided than those to red light; the average reaction time for the head and for the tail was about the same.

No experiments were tried to determine the effect of red or blue light upon the ventral side of the body, either with *Cryptobranchus* or with *Necturus*.

That it is not the organs of the lateral line system that respond to these light stimuli seems probable, at least in *Cryptobranchus*, from the fact that the head region, which is most abundantly supplied with the organs, is less sensitive to light than is the tail. In *Necturus*, when the light came from above, the head gave the most sudden responses; but when the light was from below the tail was more responsive than the head. This would seem to indicate that the more rapid response from the head was due to the sensitiveness of the eyes, though why this should be the case with *Necturus* and not with *Cryptobranchus* is not apparent.

Parker ('05) thinks that the sensitiveness to light of young lamprey eels is due to stimulation of the ends of the spinal nerves in the skin; this may be the case here also. He found ('05) that in the ammocœtes, as in *Cryptobranchus*, the tail is the most sensitive part of the body, the response being negative. In the frog, on the other hand, he found ('03) the reaction to be positive. Just as it is of value to the young lamprey, on account of its burrowing habits, to have a tail that is sensitive to light, so is the sensitive tail probably of value to the giant salamander because of the animal's habit of concealing itself under objects in its native haunts. The fact, as I have already shown ('05), that the eyes of this animal are probably not very sensitive, may have something to do with the unusual sensitiveness of the tail.

Thermic Reactions of Cryptobranchus.

The temperature experiments were made upon four adult specimens, the same ones that were used in the light experiments.

When taken from their tank, where the water was at 18° C., and put into water at 33° none of the four animals showed any signs of being aware of the change of medium; the same negative results were noted when they were changed from water at 5° to water at 26°. On one occasion, however, they showed slight signs of discomfort when put from water at 14° into water at 26°.

Removal of the animals from water at either 18° or 26° to water at 42° resulted in the most violent struggles, beginning after an immersion of two or three seconds and lasting, usually, for a minute or more, or until the animal was completely exhausted. The violence of these struggles was quite remarkable, though less marked in some cases than in others. In some instances the struggles continued until the animal was completely exhausted and turned belly side up as though dead; in other cases the struggles gradually ceased until the animal lay quietly in the warm water. Two of the animals died soon after the experiments, probably as the result of their experiences, as they had seemed perfectly healthy before.

Removal from water at 26° to water at 5°, or from water at 18° to water 0° caused no reaction, although, in the former case,

there was a decrease in temperature of 21°. Neither did an increase of 21° or more cause any reaction unless the higher temperature reached 40° or more. It would seem, then, that *Cryptobranchus* is not sensitive to considerable changes in temperature, but is very seriously affected by a temperature only slightly higher than that of the human blood.

Thermic Reactions of Necturus.

As might be expected, perhaps, from its having gills, *Necturus* proved to be more sensitive to temperature variations than was *Crytobranchus*.

When transferred from water at 0° C. to water at 18° three of the five animals showed little or no reaction, but the other two darted about the tank in a curious, spasmodic way quite different from their usual motions. When transferred from water at 18° to water at 32°, three of the animals again showed but little activity, while the other two struggled quite violently. When put into water at 42° from water at 32° or lower, all of the animals struggled as violently as did *Crytobranchus*.

When put into water at 4° from water at 32° two of the animals again showed marked activity, while the other three were scarcely affected. A very short stay in the warm water was sufficient to completely exhaust all of the *Necturus*, so that they turned belly side up, but when returned to water of moderate temperature they soon recovered.

Necturus is apparently sensitive to considerable changes in temperature, both rising and falling, as well as to the higher temperatures to which *Crytobranchus* responded.

Other and more accurate experiments along these lines suggested themselves, but on account of the lack of the necessary facilities they had to be postponed.

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NOTES ON BRANCHIOBDELLA.

W. M. SMALLWOOD.

So little is known concerning the American members of *Branchiobdella* that the following notes may be of interest. During the summer of 1905 a number of crayfish were taken by dredging from Lake Clear, Harrietstown, Franklin County, N. Y., in about eighteen feet of water. On all of the claws the carapace, rostrum, and even on the abdomen there were large numbers of *Branchiobdella instabilia* Moore which Professor Moore has kindly identified for me. I found associated with them a few *Branchiobdella pulcherrima* and a half dozen *Bdellodrilus illuminatus*.

All of these are usually designated as external parasites but I can see no reason for using this term. The word symbiosis or commensalism more correctly describes the relation that exists between the crayfish and *Branchiobdella;* but these two terms are used with such inconsistency to-day that we can find no accepted definition of either. It is not easy to conceive how *Branchiobdella* is of benefit to the crab. Their whitish appear ance and constant movements make the crab more conspicuous yet from their location and the fact that they are attached by the posterior end suggest the probability that they do not receive anything as food from the crab's body.

The food of these forms throws some light on their habits. A study of the digestive tube in both macerated and mounted sections revealed the presence of large numbers of unicellular algæ and diatoms. The digestive tube was full and seemed to contain nothing but small plants which would suggest that the crayfish merely served to carry the worms around enabling them the better to secure food so that it seems to me that the term symbiosis used in its original and etymological sense gives a better idea of these relations than does external parasite.

All of these symbionts are continually in motion, stretching the body out in every direction as if constantly feeling for something.

During these movements *B. instabilia* change their shape, the long, slender worm quickly becomes short and broad posteriorly. I had some small clepsine in the same aquarium and was much impressed with the great similarity of movement between the two. This peculiar leech-like movement together with the prominent posterior disk by means of which the animal adheres to the crab so simulates the general external appearance of leeches that it was doubtless one of the reasons for classifying *Branchiobdella* at first with the hirudineans.

Moore, '93, first describes these two representatives of *Branchiobdella* and reports them only from Watauga Co., North Carolina, and Delaware Co., Pa., and so far as I have been able to determine they have not been recorded as occurring farther north than Pennsylvania. The water of Lake Clear is derived almost entirely from springs, but two mountain brooks flow into it, so that the water does not warm up much during the summer. The latter part of July, when the worms were taken, the temperature of the water along the shore rarely exceeded 70° F. during the day. When the crayfish having the parasites were placed in a small aquarium the *Branchiobdella* did not live more than twelve hours although the water was taken directly from the lake and changed once during that time. I am inclined to attribute their death largely to the rise in the temperature of the water as the aquarium was placed in the boat house, which was at least 15° to 20° warmer than the water in the lake.

The animals taken all seemed to be sexually mature, as subsequent study proved, and were all about the same size. Although I have over fifty specimens of *Branchiobdella instabilia* there does not seem to be any noticeable variation in their size or appearance.

The biannulation of all but the head segment in *B. pulcherrima* and the first four just back of the head in *B. instabilia* is very suggestive of leech-like affinities in these forms, which are now generally agreed as being oligochætes. The cœlomic cavity is clearly defined and perfectly divided by dissepiments into a series of cavities. The cœlomic space is encroached upon by the enormously long muscle cells which correspond to the well-known circular and longitudinal muscles of the oligochætes.

After studying a considerable number of animals in serial sections I find but one type of muscle cell. The contractile tissue is arranged around the outside of the cell and is striated in most instances. Within the contractile region there is a granular protoplasm containing a spherical nucleus which corresponds to the ordinary nucleus found in any tissue. Voigt, '86, describes several arrangements for the contractile tissue in the European form *B. varians.*

The nervous system in both *B. instabilia* and *pulcherrima* can be easily seen in a mounted specimen and the ganglionic swellings in the latter make a very clear demonstration of their relation to somites. In section the nerve strand is seen to be clearly bilaterally symmetrical.

My chief observations are concerning the development of the sex cells and the results do not confirm all of the work done by Voigt. Voigt, '85, has given a full description of the formation of the eggs and sperms of the European *Branchiobdella varians* with 135 figures. The parts of the adult sperm as described by him are the same as those in *B. instabilia.* Concerning the development of the germ cells, however, the agreement is not so satisfactory.

The nebenkorper is unmistakably described for the nucleolus in the ovocyte on page 322. In Fig. 57 "Sind die kerne der spermatocyten noch ungeteilt; in Zweien von Zellen ist das Nebenkörperchen noch einfach, in den anderen bereits doppelt." In the figure referred to above and in succeeding ones there can be no doubt but that the nucleolus is the body described under the caption of Nebenkörperchen.

The spermatid has two nebenkörperchen, one that has persisted, the other is developed from the thicker protoplasm near the cell wall. "Das eine davon is das Nebenkörperchen, das andere besteht, wie sich herausgestellt hat aus einer Ausammlung von dichterem Protoplasma an der Zellwand, da, wo der Schwanzfaden hervorwachst und soll in folgenden als 'Bildungskörperchen des schwanzfaden' bezeichnet werden" (page 324).

In describing the parts of the adult spermatozoön the nebenkern is derived from the nebenkörperchen, and, as already cited, this latter structure persists and becomes the tip end of the

sperm. The parts of the sperm and their derivation are summarized in the following words: "Der Samenkörper besteht also jetzt (Fig. 132): (1) aus dem nebenkern, (2) dem von einer dünnen Membran gebildeten Schlauch, (3) dem asu dem Kern hervorgegangenen konischen Teil, (4) dem Verbindungsstück, welches aus dem protplasma der Zelle anstand und aus welchem, (5) der Schwanzfaden hervorgesprosst ist," page 328.

Moore, '95, describes the morphology and histology of *Bdellodrilus* in detail, a form which was first described as *Branchiobdella*, and the agreement of *B. instabilia* is so close that a separate description is not warranted. In connection with the description of the reproductive system the following statement is made: "The first steps in the development of spermatozoa begin before the worm has nearly reached full size, and proceeds continuously; the various stages floating freely in the cœlom, in which they complete their development. In the mature worm the cavities of the fifth and sixth post-cephalic somites are filled with spermatozoa in various stages of development, while the testes proper have become much reduced and inconspicuous. The details of this process have been admirably worked out and described by Voigt; and it need only be added that what observations the writer has made are in accordance with his account" (page 519). In the same year Calkins' paper appeared on the spermatogenesis of *Lumbricus* and makes no mention of the works of Voigt.

The ovaries are located in the seventh post-cephalic somite and consist of two separate sacs, one on each side of the digestive tube. The young ova in one half of the ovary may be in a state of division while the rest of the ova are in the resting state. This division is by the usual indirect process with no evidence of astral fibers. A distinct centrosome is present at each pole of the spindle, the spindle fibers arising from within the nucleus. The chromatin assumes the characteristic spireme state preceding the formation of the equatorial plate. An early prophase stage in the division of an ovum is shown in Fig. 5. Voigt figures ova having two nucleoli and believes that this is an indication of amitotic division. My sections have been studied with this particular point in mind and no case of amitosis has been noted. In a few

instances ova containing two nucleoli (Fig. 4) were found. The nucleolus is large and when mitosis begins has undergone a transformation similar to the changes described for *Haminea* (Smallwood, '05). This would indicate that the nucleolus breaks up and does not take an active part in division. Furthermore, in some cells the two centrosomes in the prophase were observed while the nucleolus was still present, the difference in size is alone sufficient to prevent confusion; Voigt has apparently confused them in describing the process of direct division.

As the eggs mature, they are found on the surface of the ovary and can be readily distinguished by their large size as compared with the surrounding young ova. Associated with the ovary on one side only in most instances, there is found a large mass which is apparently nutritive in character (Fig. 3). An attempt was made to detect the beginning of this modification. The membrane which surrounds the ovary is continuous and passes around the nutritive mass. This structure extends from the extreme dorsal part of the cœlome of the seventh somite to the

FIG. 1. A photograph of *Branchiobdella instabilia* contracted.

ventral body wall (Fig. 3). Several sections show this mass even passing beneath the intestine. Frequently the neural sheath of peritoneum can be shown to be continuous with the sac enclosing this mass and the eggs. All of the masses studied had a faint nucleus and in one instance a nucleolus was found which would

seem to indicate that this mass was a very much enlarged cell. One animal showed a few small ova in the edge of this mass adjacent to the ovary but in all other animals there was a clearly defined outline between the ova and this mass, which indicated unmistakably that the ova cells were in the ovary. This nutritive mass shows two clearly defined regions, a dorsal and ventral. The dorsal portion contains many small round bodies very uniform in size in a protoplasmic matrix. These bodies take a basic stain while Bordeaux red colors the matrix ; the ventral region does not contain these bodies, nor is it composed of granular protoplasm but yet it takes a basic stain. Its appearance in the fixed state suggests that it exists as fluid in the living animal. When the egg of the hydroid *Clava* is stained as above and viewed with the oil immersion lens, the conditions are so nearly identical that unless one knew in advance I very much doubt his ability to distinguish the two as belonging to different species. It is interesting to find this similarity in two such widely separated animals and it also helps to interpret the conditions in *Branchiobdella instabilia*. These bodies are interpreted as nutritive by Hargitt,[1] probably of a proteid nature. In *Branchiobdella*, I am inclined to believe that this nutritive mass is discharged into the cocoon where it nourishes the growing worms.

Voigt finds the beginning of what he designates a degeneration process. This process consists in the deposition of fat-bodies in certain ova but no such large size is reported nor is the change limited to one cell. Of course one can not be sure that but one cell has taken part in the formation of this large nutritive cell for the process may involve the growth of one cell at the expense of many others as is so often the case in the growth of eggs. But that this change is a fat-forming process is much to be doubted as no fat reactions were obtained. It is probably rather a normal growth process, the nutrition being stored in this special cell rather than in the cytoplasm of each egg.

What has already been said for the appearance of the ovary and manner of division may be repeated for the cells in the testes. In size, shape, reaction to stain, and appearance, they are

[1] Hargitt, C. W., "The Organization and Early Development of the Egg of Clava leptostyla," in press, BIOL. BULL.

very similar. The testes are usually somewhat larger than the ovaries and contain more cells. They are located in the sixth post-cephalic somite. Frequently the cells in the testis are pear-shaped as contrasted with the spherical form of the young ova.

FIG. 2. A photograph of *Branchiobdella pulcherrima* showing the post-cephalic segments, the digestive tube and ganglionic swellings. In the sixth post-cephalic segment the two testes are on one side and in the seventh the ovary shows.

No evidence was observed to show that nutritive bodies such as Voigt reports for *B. varians* were present in the cytoplasm; nor is there a special nutritive cell as in the case of the ovary. In *Branchiobdella pulcherrima* the two testes were found on the same side in one animal (Fig. 2).

Associated with the formation of the male sex cells from the time they leave the testes until the spermatozoön becomes fully grown there is a protoplasmic structure, termed the blastophore (Bloomfield, '80, Calkins, '95) in *Lumbricus*, and the cytophore (Voigt, '85) in *Branchiobdella varians*. These two terms seem to refer to the same structure and the more recent term, blastophore, will be used in this paper.

Calkins traces the formation of the blastophore and finds that it appears before the sperm cells leave the testis. While still in the testis he figures the blastophore as consisting of eight nuclei in an undifferentiated protoplasm. Repeated division of the nucleus unaccompanied by the formation of cells gives rise to a multinucleate cell. After a time "cytoplasmic cleavage occurs

around each nucleus, thus differentiating the blastophore from the germ cells" (p. 275). In *B. varians* (Voigt, p. 314 sq.) the blastophore arises in a similar although not identical manner. It is first seen just as the spermatogonia escape from the testis at which time but two or four cells are associated with it. When the spermatogonia divide, a portion of the protoplasm is not enclosed within the cell wall, although it is directly connected with the cytoplasm of the cell. The young stages show the blastophore more or less irregular in shape, but as the cells become more numerous it is uniformly spherical. It differs in two particulars from the conditions in *Lumbricus*: (1) it is found in an earlier stage in the cœlome; and (2) the spermatogonia cells are always distinct in outline from the blastophore, while the earlier stage in *Lumbricus* is more like a syncytium.

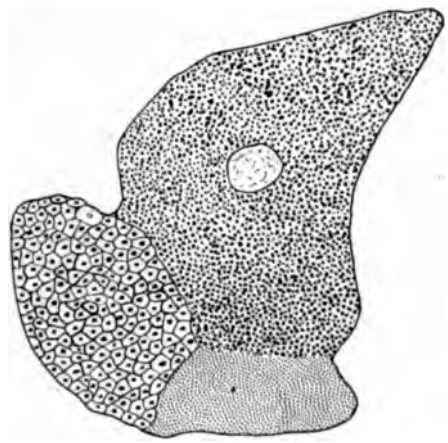

FIG. 3. A camera drawing of a single section of the ovary and nutritive mass. The dorsal granular portion contains a loosely organized nucleus. The ventral non-granular portion is larger in the other sections of it.

Branchiobdella instabilia repeats in the formation of the blastophore the conditions of *B. varians*. During the division of the spermatogonia the plane of division has a definite relation to the center of the blastophore. A definite spindle is always present with its long axis tangential to the surface of the blastophore, so that a plane passing through the equatorial plate, if continued, would go through the center of the blastophore. When the

spermatogonium divides, the division does not completely separate the two cells, but leaves them in connection with the blastophore. Each cell surrounding the blastophore has a direct protoplasmic connection with all of the other cells. Voigt finds as many as 128 cells surrounding the blastophore. One hundred cells were located with the camera lucida on the upper half of the blastophore of *Branchiobdella instabilia*, which would give about two hundred cells in all. The whole structure is so small that an accurate account is practically impossible. But from this estimate we can get some idea of the number of generations of spermatogonia and spermatocytes.

As the spermatid elongates, it remains attached to the blastophore by the tip end of the head and continues so connected until the spermatid has become a full-sized spermatozoön. The blastophore becomes reduced in size during this growth, vacuoles appear in it and the plasma stains give a more marked reaction. The reduction in size is so great in some instances that the blastophore is no larger than the leucocytes, but can be distinguished from them because of its non-nucleated condition. I believe that the blastophore is nutritive in character because of the origin and attachment of the developing spermatids and the accompanying degeneration in it as the spermatozoa approach full size (Calkins, p. 276), concludes his discussion of the blastophore with the following statement: "It lives as long as the developing germ cells are connected with it and dies when deserted by the spermatozoa. Nor is there any reason to suppose that it provides nutriment for the spermatid cell."

The phenomena of reduction are not readily determined in *Branchiobdella instabilia*. Continued search has been made to ascertain the number of chromosomes and at what stage the reduction occurred but thus far I have been unable to satisfy myself or to secure constant results. The size of the cells shown in Figs. 5 and 6 is the main reason for my failure. A study of the karyokinetic division of the cells surrounding the blastophore shows that the spindle fibers arise from the nucleus and terminate in a definite centrosome at each pole. No astral rays are present. The centrosomes lie close to the nuclear wall which gradually breaks down (Figs. 5 and 7).

As the spermatocytes become transformed into the spermatid, the chromatin gradually changes from the reticulate state into a compact, homogeneous mass. The spermatid is a small oval-shaped cell attached by the tip end of the head to the blastophore. Between the nucleus and the cell wall in the region farthest from the blastophore, there is a body which takes a basic

FIG. 4. A camera lucida drawing of an ovocyte with the No. 6 comp. ocular and 2 mm. objective.

FIG. 5. Prophase of young ovocyte drawn to the same magnification as Fig. 4.

FIG. 6. Metaphase of spermatocyte No. 12 comp. ocular and 2 mm. objective.

FIG. 7. Metaphase of spermatocyte showing the relation of the spindle to the nucleus, drawn to the same magnification as No. 4.

stain and is the centrosome or centrosomes of the last spermatocyte generation. The cells become so very small and the cytoplasm so much reduced that one can not be certain that there are two bodies; at this stage the body is much larger than the centrosomes during mitosis.

The distal portion of the cytoplasm becomes drawn out and forms the tail, at the same time the sperm centrosome elongates forming a connection between the nucleus and tail. This region becomes the middle piece in the adult sperm. The rapid elongation of the tail and nucleus soon renders it impossible to detect the presence of the cytoplasm. During the elongation of the nucleus, it stains more intensely and becomes constricted as if a large number of shallow parallel furrows crossed it at about 90° to the long axis of the sperm. The very tip of the sperm during nearly all of these changes remains in connection with the blastophore.

From the quotations cited from Voigt's interpretations of the spermatogenesis of the European *Branchiobdella*, it is apparent that there are important differences. In a few instances a structure which I regard as the nebenkern as described by Calkins (p. 289) was seen but this structure had no relation in its mode of origin to the nucleolus nor does it play an important rôle in

subsequent development. Voigt believes that he can trace the nebenkörpenchen from spermatogonium, through spermatocyte and spermatid to the apex of the sperm, which I very much doubt. What he has described and figured for the nebenkörpenchen in some of these early stages, I have frequently found in the free cells in the cœlome which are undoubtedly leucocytes. Kukenthal ('85, p. 454) who has made a study of the lymphoid cells of Annelids states that "The fluid of the cœlome is not homogeneous, but contains a number of elements of various kinds, among which the rather large rounded cells are the most conspicuous ; these are the lymphoid cells, and of them there are two kinds. Some have more or less finely granular protoplasm, and others contain clear highly refractive granules, which are colorless ; others are still larger, and of a yellowish-brown color." Voigt states that the nebenkörperchen can only be seen in the free living cells in some stages which is an additional confirmation of my criticism.

The statement that the spermatid has two nebenkörperchen, one of which is derived from the thickened protoplasm of the cell wall, is of course due to his inability to detect the origin of this body in the spermatocyte centrosome. The manner in which the nucleus is supposed to form the head of the sperm is somewhat unique. The head of the sperm grows out of the nucleus much as the radicle grows out of the bean, the nucleus becoming smaller as the head lengthens instead of the complete and simultaneous transformation of the whole nucleus into the head.

Branchiobdella instabilia is so closely related to *Branchiobdella varians* that the spermatogenesis of the two species is probably very nearly identical and a restudy of the latter will probably show its full agreement with the former as well as with other annelids.

SYRACUSE UNIVERSITY, DEPARTMENT OF ZOÖLOGY,
April 15, 1906.

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BIOLOGICAL BULLETIN

THE RELATION BETWEEN REGULATION AND FISSION IN PLANARIA.

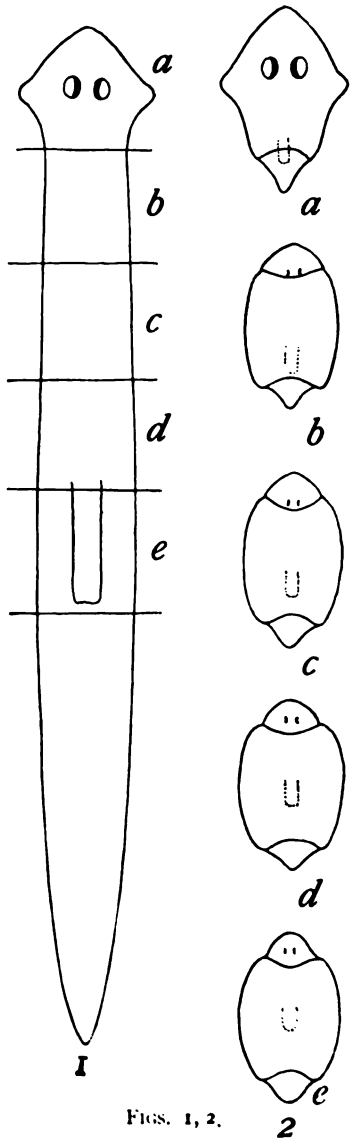
C. M. CHILD.

The data discussed below were obtained at various times during the last five years from two species of *Planaria*, *P. maculata* and an unnamed Californian species differing from *P. maculata* in color, length of "auricles" and length of pharynx (Fig. 8), but resembling it very closely as regards regulation and fission.

I. THE POSITION OF THE PHARYNX IN PIECES FROM DIFFERENT LEVELS.

It has been known since Morgan's experiments on *Planaria maculata* that the position of the newly formed pharynx in a piece differs according to the level of the body from which the piece was taken. According to Morgan the new pharynx arises posterior to the middle of the piece in prepharyngeal pieces, its distance from the middle decreasing as the level of the piece approaches the pharyngeal region of the original animal; as regards postpharyngeal pieces, however, he states merely that the pharynx arises anterior to or near the middle of the piece. My own observations on prepharyngeal and pharyngeal pieces agree with his. Figs. 1 and 2, *a–e*, indicate this relation between level and pharyngeal position. The regulation of postpharyngeal pieces, however, presents a number of important features, which Morgan has not, so far as I am aware, described. If the whole postpharyngeal region be cut off as one piece, the new pharynx always arises a considerable distance anterior to the middle (Fig. 3) its position varying somewhat in different cases. If, however, this region be cut into several pieces (Fig. 4, *a*, *b*, *c*, *d*)

we find that the new pharynx appears anterior to the middle in pieces from the anterior part of the postpharyngeal region and at or very near the middle in pieces from the posterior portion (Fig. 5, *a*, *b*, *c*, *d*); in other words *the distance between the new pharynx and the anterior end increases with increasing distance from the old pharyngeal region.* By analogy with the prepharyngeal region we should expect just the opposite, but in only two cases out of more than a hundred have I seen it (Figs. 6 and 7, *a*, *b*, *c*). In these two cases *the distance between the new pharynx and the anterior end of the piece decreases with increasing distance from the old pharyngeal region.*



FIGS. 1, 2.

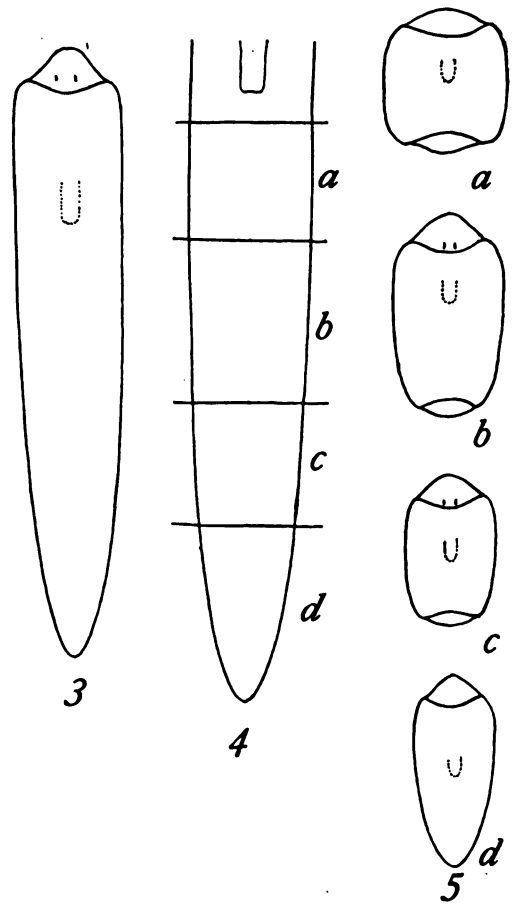
It is necessary here to refer very briefly to certain interpretations suggested in previous papers.[1] According to these suggestions the course of regeneration in a piece will depend very largely upon its past associations with the various functional

[1] "Studies on Regulation — IX., The Position and Proportions of Parts During Regulation in Cestoplana in the Presence of the Cephalic Ganglia," *Archiv f. Entwickelungsmech.*, Bd. XX., H. 1, 1905.

"Studies on Regulation — X., The Positions and Proportions of Parts During Regulation in Cestoplana in the Absence of the Cephalic Ganglia," *Archiv f. Entwickelungsmech.*, Bd. XX., H. 2, 1905.

"Contributions toward a Theory of Regulation — I., The Significance of the Different Methods of Regulation in Turbellaria," *Archiv f. Entwickelungsmech.*, Bd. XX., H. 3, 1906.

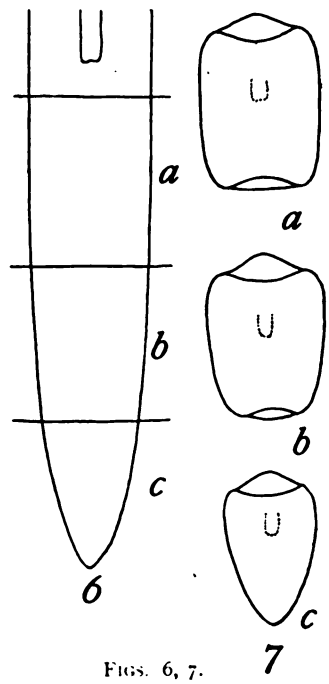
complexes of the whole, thus a piece from the anterior regions of the body is functionally much more "anterior" than a piece from the middle of the body; consequently in the new wholes formed from these pieces we shall find relative differences in size of various parts. In the "anterior" piece, for example, the anterior region will be much larger than the posterior and in the piece from the middle of the body the two will be about equal.



FIGS. 3–5.

In normal animals the pharynx is situated in the middle of the body but in those new animals formed from the pieces the physiological or functional middle does not coincide with the

structural middle: in anterior pieces the functional middle is near the posterior end; in pieces from the middle of the body it is near the middle, etc. The new pharynx appears at this functional middle or as we may more properly call it the physiological pharyngeal region. With this interpretation in mind the difference in position of the pharynx in the pieces *a–c*, Fig. 2, is readily understood.



Figs. 6, 7.

Now we might expect that pieces from the postpharyngeal region would show relations similar in character but reversed, *i. e.*, a decrease in size of the new prepharyngeal region with increasing distance from the pharynx. As a matter of fact just the opposite occurs (Figs. 4, 5, *a*, *b*, *c*, *d*) except in very rare cases (Figs. 6 and 7) so that the prepharyngeal and postpharyngeal regions are usually equal in size in pieces from the extreme posterior end.

We must conclude from this relation that pieces such as *c* and *d* in Fig. 5 are physiologically more anterior than pieces *a* and *b*. I believe that the occurrence of fission affords a very simple explanation of these facts. It is highly improbable that fission occurs in these forms without some physiological preparation even though changes in structure may not be visible. Various authors have noted that fission in certain other turbellaria is initiated by changes in the nervous system leading to the development of new cephalic ganglia. If such changes are going on in this postpharyngeal region in *Planaria* there is no difficulty in understanding why the new prepharyngeal region should be longer in these pieces than in those farther anterior. Moreover,

I do not believe that any other explanation of these remarkable facts can be found.

The two exceptional cases noted above (Figs. 6, 7, *a*, *b*, *c*) were from worms captured through the ice in January when food was scarce and activity slight on account of the low temperature and hence in all probability no preparation for fission existed. These cases only serve, therefore, to render more probable the interpretation given above.

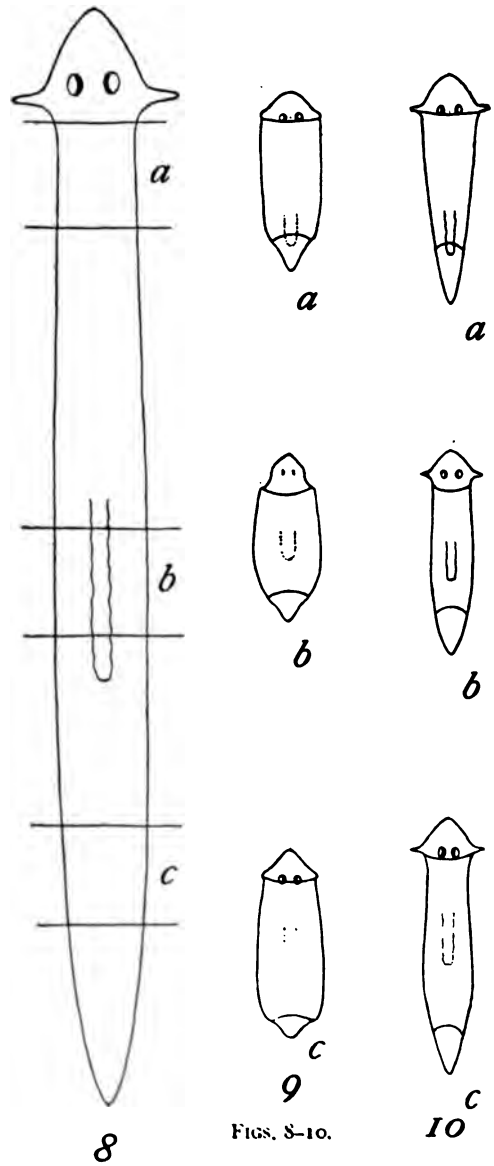
In *P. simplicissima*, which is not known to undergo fission the relative position of the pharynx in pieces as from different levels is, according to Morgan, similar to that in the winter specimens of *P. maculata*.

II. The Size of the Head at Different Levels.

In 1901 I first noticed that the new head formed was larger in pieces from the anterior part of the prepharyngeal region and from the posterior half or two thirds of the postpharyngeal region than in pieces from the middle region of the body. Moreover, in the pieces from the middle region a considerable amount of new tissue is formed posterior to the eyes while in the anterior and posterior regions the eyes lie almost in the plane of the cut surface.

If we cut the whole body into equal pieces and compare them we find the size of the head decreasing and the distance between the eyes and the cut surface increasing as we proceed posteriorly toward the old pharyngeal region. Pieces just anterior or posterior to or in the pharyngeal region are much alike, all forming relatively small heads and relatively long "necks" of new tissue. In the postpharyngeal region, however, the size of the head increases and the length of the neck decreases until in the posterior half of the postpharyngeal region the head is about as large as in anterior pieces and the eyes appear in the plane of the cut. Both species of *Planaria* are similar in this respect. Figs. 8, 9 and 10, *a*, *b*, *c*, illustrate the case under consideration. Fig. 8 indicates the regions from which the pieces *a*, *b* and *c* were taken; in Fig. 9, *a*, *b*, *c*, the difference in size of the head and length of the "neck" in the new tissue is shown at an early

stage of regulation and in Fig. 10, *a*, *b*, *c*, at a later stage. These figures as well as all my conclusions on this point are based on



FIGS. 8–10.

careful and repeated measurements of all pieces, although the difference is sufficiently striking without such aids.

I believe that these differences are also indicative of the physiological conditions at different levels. In previous papers[1] I have suggested that the relation between redifferentiation and regeneration proper depends upon the physiological differentiation of parts. As the difference in physiological or functional specification between the part removed and the part remaining increases the amount of redifferentiation decreases and that of regeneration increases up to a certain limit and vice versa. To take a concrete case: the more "head-like" in the physiological sense the anterior portion of a piece of *Planaria* is, the smaller the amount of new tissue formed at this end and the greater the amount of redifferentiation. Now the anterior ends of pieces from the anterior regions of the body of *Planaria* and those from the posterior half of the postpharyngeal region are visibly more "head-like" than those of pieces from the middle region. This is evident from the degree and character of their activity. Correspondingly we find that only the part anterior to the eyes is formed by regeneration, the "neck" and in some cases the auricles themselves being formed by redifferentiation in the pieces from anterior and posterior regions (Figs. 9, *a*, *c*, and 10, *a*, *c*). On the other hand, in the pieces from the middle region not only the head but a longer or shorter "neck" is formed by regeneration.

The difference in size of the heads furnishes more evidence along the same line. The greater the relative intensity of a given functional complex the larger the relative size of the structural complex which represents it. Hence the more "head-like" a given piece is the larger the relative size of the head formed and vice versa (cf. Figs. 9, *a*, *b*, *c*, and 10, *a*, *b*, *c*).

The conclusions from this line of evidence are the same as those reached from a comparative study of pharyngeal position *viz.*, that in the posterior half or two thirds of the postpharyngeal region a marked increase in the functional conditions charac-

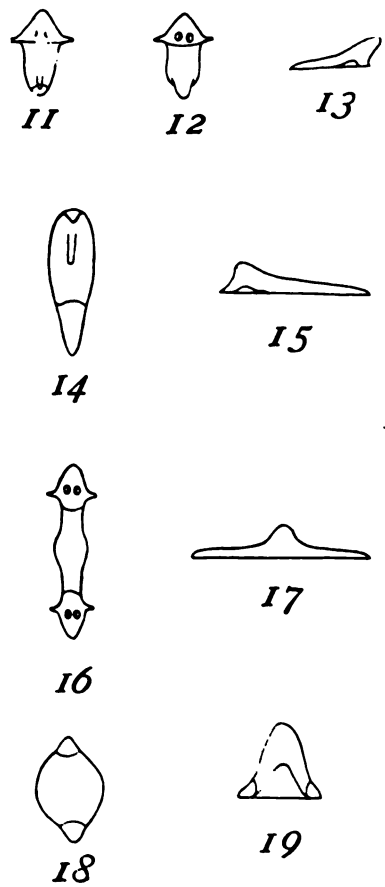
[1] "Studies on Regulation — VIII., Functional Regulation and Regeneration in Cestoplana," *Archiv f. Entwickelungsmech.*, Bd. XIX., H. 3, 1905.

"Contributions toward a Theory of Regulation — I., The Significance of the Different Methods of Regulation in Turbellaria," *Archiv f. Entwickelungsmech.*, Bd. XX., H. 3, 1906.

teristic of the anterior region occurs — that is the change is due to the development in this region of a new "head" physiologically speaking.

III. Regulation in Very Small Pieces.

By the use of pieces approaching the minimal size in which regulation is possible, we have a means of determining the physiological potences of the various regions more exactly than by any other method. I have recorded the full history of a large number of species of this kind from both species of *Planaria*, though mostly from the Californian.



Figs. 11-19

Worms of rather large size were selected and cut into eighteen to twenty pieces by transverse or nearly transverse cuts. Each piece was numbered and isolated and observed as an individual. The results of these experiments are rather remarkable. The pieces from the region immediately posterior to the old head produce a large head and a very small posterior end with the pharynx near the latter (Fig. 11) or sometimes no pharynx, *i. e.*, these pieces are so exclusively "anterior" physiologically speaking that sometimes they are incapable of giving rise to the pharyngeal and postpharyngeal regions at all, and in any case these regions are small. The pieces behind these show a smaller head and larger posterior end with the pharynx approaching the middle and give rise to normal animals. But a region including the old pharynx and extending for a short distance anterior and posterior

to it affords a remarkable variety of results in these small pieces. Five different methods of regulation occur: (1) The piece may form a normal animal; (2) it may be fully used up in the formation of a head and anterior region without a pharynx or postpharygneal region (Fig. 12). In such cases a dorsal thickening occurs in the posterior region (Fig. 13, optical section in median plane); (3) the piece may go entirely to the formation of a posterior region without head and with or without pharynx (Fig. 14). Such pieces bear a dorsal thickening in the anterior region (Fig. 15); (4) the piece may form a head at each end (Fig. 16). In these cases there is a dorsal thickening in the middle (Fig. 17); (5) the pieces may form a tail at each end (Fig. 18). Such pieces acquire after two or three weeks the outline shown in Fig. 19 in consequence of the opposed activity of the two posterior ends.

The cases 3 and 4 have been described by Morgan and others but so far as I am aware cases 2 and 5 have not been recognized as what they actually are, though they have undoubtedly been seen by many observers and grouped with abnormalities. The pieces from the postpharyngeal region almost without exception produce normal animals. Occasionally a case of double heteromorphic heads like Fig. 16 occurs very near the extreme posterior end of the body, often in next to the last piece.

Thus the middle region as compared with the anterior and posterior regions appears to be indifferent; pieces from it may form single or double anterior parts, or single or double posterior parts, or normal animals. The different possibilities do not occur in any definite order; a single or double "tail" is as likely to follow a double "head" as is a normal animal and *vice versa*. Neither does the presence of the old pharynx play any part in the production of these various results, for the region where they occur most frequently begins some distance anterior to the pharynx and ends some distance posterior to it.

I believe that we must consider this region as physiologically indifferent, *i. e.*, neither "anterior" nor "posterior" in the functional sense to any marked degree. In consequence of this indifference the actual course of regulation must depend on slight chance internal differences in the pieces. If one end becomes pbysiologically dominant the piece may give rise to a single

"head" or a single "tail." If both ends maintain their independence a normal animal or double "heads" or double "tails" may result according as the physiological conditions are different or alike. The dorsal thickening in these cases of partial animals represents that region of the body toward which the intestinal contents are forced during contraction. It is caused by the formation of an intestinal cavity in this region in consequence of the pressure and this cavity continually enlarges in the direction of least resistance, *i. e.*, directly dorsally (Figs. 17 and 19) or dorso-posteriorly (Fig. 13) or dorso-anteriorly (Fig. 15) as the case may be. As the size of the pieces from this region increases normal animals are more frequently formed until beyond a certain limit all pieces give rise to normal animals.

At present I desire chiefly to call attention to the fact that the two regions separated by this indifferent region resemble each other in regulation since they usually produce normal animals. We might expect from their position in the body to find the pieces from the post-pharyngeal region predominately "posterior" in function but as a matter of fact they show "posterior" and "anterior" characteristics in equal degree since prepharyngeal and postpharyngeal regions are of the same size. Here again the only satisfactory explanation of the facts is the existence of the early stage of individualization of a new head region somewhere in this postpharyngeal region. The results with small pieces indicate that this condition is not sharply centralized or localized in the region but extends more or less completely throughout its length. Moreover, the remarkable agreement of these different lines of evidence when considered from the point of view adopted in this paper affords an indication of the value of this point of view as a means of interpretation and unification of the mass of data which have accumulated in this field.

I believe that the supporters of theories of regulation based on formative substances or upon "entelechies" will find a certain amount of difficulty in interpreting some of the facts cited here, but discussion of my conception of their bearing is postponed.

As is well known, fission in *Planaria maculata* occurs in a region some distance posterior to the pharynx and the same is

true of the Californian species. My observations have led me to believe, however, that the level varies to a considerable extent. The direct cause of separation seems to me to be a difference in reaction of the two parts; a certain portion of the postpharyngeal region attaches itself firmly while the other parts of the body attempt to move forward. Since the motor power for the forward movement, *i. e.*, the cilia and the margins of the body — is applied not at the anterior end only but throughout the whole length of the part anterior to the attached region the greatest strain will occur just anterior to the attached region and rupture will occur here even in the absence of any preformed zone of weakness. Personally I am inclined to doubt the existence of any such zone of weakness, but I think the facts show very clearly that under ordinary conditions *Planaria* consists physiologically of two zoöids.

HULL ZOÖLOGICAL LABORATORY,
UNIVERSITY OF CHICAGO,
April, 1906.

THE ORIGIN OF THE ORGAN-FORMING MATERIALS IN THE FROG'S EMBRYO.

T. H. MORGAN.

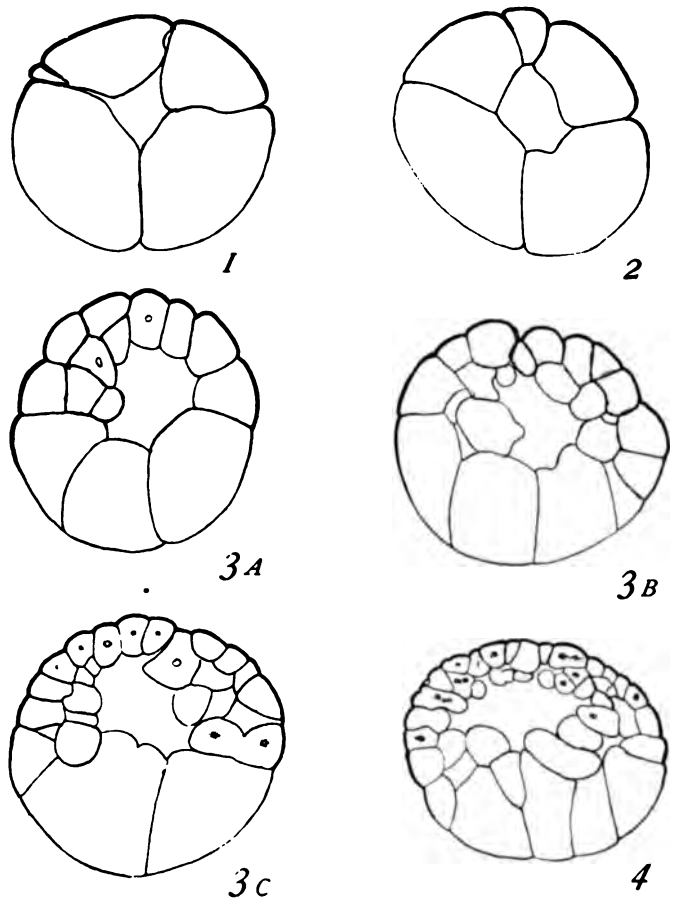
The location of the neural plate of the frog's egg, and of the organs lying immediately below it — the "embryo" in a narrow sense — has been variously determined by different embryologists. Pflüger, Roux, Schultze, O. Hertwig, Morgan, Brachet, H. V. Wilson, Assheton and others have considered this question, but since the literature has been sufficiently reviewed in recent years I shall refrain here from further comment. In the present communication I shall deal with a problem somewhat different from the location of the embryo, namely, the origin of the materials out of which the "embryo" is formed. If I can make good my point it will be seen that the embryo-forming material originates from a part of the egg different from that in which the embryo first appears, hence the location of the embyro on the egg does not give a sufficient answer to the problem of the real source of the materials out of which the embryo develops.

In a series of papers, dealing with the relation between normal and abnormal development of the frog's egg, I was led to certain conclusions in regard to the origin of the organ-forming substances, and in the last paper of the series I dealt directly with this question, basing my conclusions upon a reëxamination of normal development of *Rana palustris*. The present paper deals with the same problem in *Rana sylvatica* and *Bufo lentiginosus*, and includes some new observations on *Rana palustris*.

Argument.

Briefly stated, my view is as follows: The material, out of which the "embryo" develops, lies in the upper hemisphere, and is transported below the equator of the egg during the segmentation stages. This material forms a ring around the lateral wall of the segmentation cavity, at first above, later below the equator of the egg. The same material can be traced with some prob-

ability to the eight-cell stage, where it exists partly in the upper four cells and partly in the upper ends of the larger lower four cells. In the later segmentation stages, and in the pregastrula stages, this material moves down around the sides of the egg until it comes to lie some distance below the floor of the segmentation cavity. Here it becomes, as described above, the germ-ring, which closing over the lower hemisphere produces the "embryo."

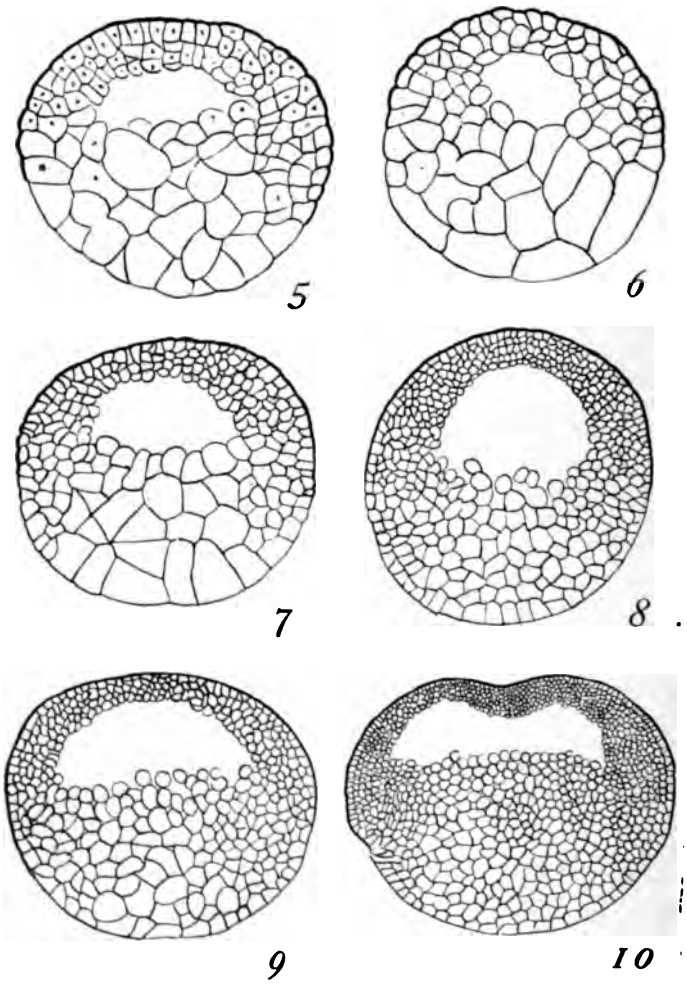


FIGS. 1–4. Segmentation stages of the *Rana palustris*.

The details of these changes, and the evidence on which these statements rest, may now be considered more fully.

Descriptive.

Rana palustris. — Since I now have a more complete series of stages of the eggs of this species, and since I have elsewhere especially considered this form, it may be dealt with first.



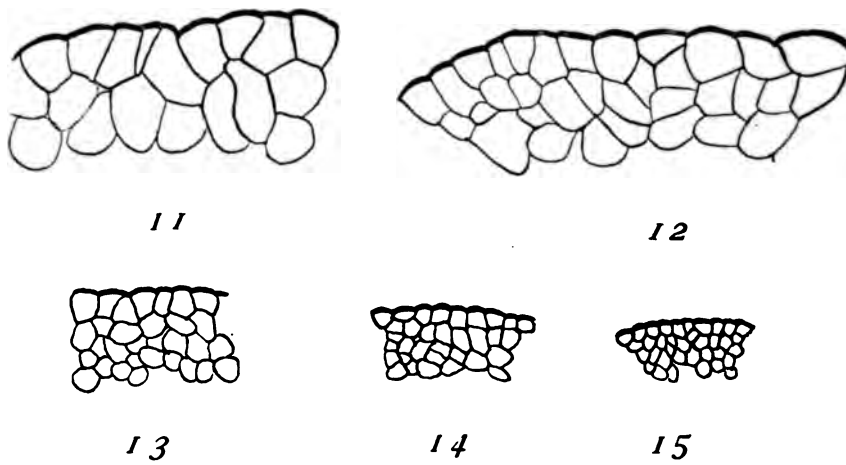
Figs. 5-10. Later segmentation stages and gastrula stage (Fig. 10) of *Rana palustris.*

Fig. 1 is a vertical section of an eight-celled stage. The segmentation cavity is present and is bounded above by the upper four cells (of which two are seen in the section), and at the sides

by the upper ends of the lower four cells. The great thickness of the upper four cells that form the roof of the blastocœl is especially to be noted. The next section, Fig. 2, is a section through a 16-cell stage. The same relations noted for the last stage hold here also. The next three figures are from another set of eggs. Fig. 3, *A*, is a 54-cell stage with six of the cells inside. The cells of the roof have already begun to move out towards the sides, so that the middle part of the roof is much thinner than that of the sides. The next figure, Fig. 3, *B*, is from an egg containing sixty-four cells, of which fifteen are inside. It shows changes similar to those of the last figure. Fig. 3, *C*, is through an egg with 105 cells, of which twenty-six are inside. The shifting of the cells derived from the upper four cells is still more apparent. At this time the top of the roof of the segmentation cavity is much reduced in thickness, while the sides are still quite thick. In the last three figures, the segmentation cavity is very irregular in shape. At first it is deeper in a vertical plane, but in the last stage it is broadening out in a horizontal plane. The last section, Fig. 4, is from an egg belonging to the same set as Figs. 1 and 2. The following six figures, Figs. 5 to 11, belong to still another series.

The fluid in the segmentation cavity is formed by the surrounding cells, and it may be supposed that the thinning out of the roof of the blastocœl is due to loss of fluid, but as the egg as a whole slowly increases in size during this time, the loss of fluid to the blastocœl must be made good by the absorption of water from outside. During the following stages, as seen in Figs. 5–9, the roof becomes very much thinner, as shown strikingly on comparing the sections of the roof at different stages, as seen in Figs. 11–15. It will also be noticed on comparing sections of the earlier and the later segmentation stages, that the side-walls of the segmentation cavity are at all stages very thick compared with the upper wall, and also that the smaller cells of this region are being slowly carried down at the sides of the egg. They furnish the material out of which the "embryo" is later formed. In Fig. 10 the first indications of the dorsal lip are present, as seen to the left. At this stage the smaller cells have pushed down below the equator of the egg on the side at which

the dorsal lip of the blastopore lies. At the same time the lateral wall of the blastocœl has been reduced in thickness, and there can be little doubt that the reduction must be directly due to the movement downwards of its cells. On the opposite side of the section where the ventral lip will form later the down-growth of small cells is less marked, and the lateral wall of the blastocœl is correspondingly thicker. In other words, the ring of embryo-forming material lies at this time obliquely on the egg one side having pushed down further than the other. On the right and left sides of the embryo, as seen when cross-sections are made, the ring shows all intermediate stages between the condition at the dorsal and at the ventral lip.



FIGS. 11–15. Roof of segmentation cavity of *Rana palustris*.

The later history of the gastrulation has been sufficiently described in former papers. In general, the process consists of the steady migration of the embryonic ring over the lower hemisphere. As this takes place the yoke is thrown high up into the segmentation cavity, and the roof of the latter becomes reduced to a thin layer of cells.

The steady decrease in thickness of the roof of the segmentation cavity during the cleavage, pregastrula and gastrulation stages is a constant feature of the development. The decrease in thickness is well shown in the series of figures, Figs. 11–13,

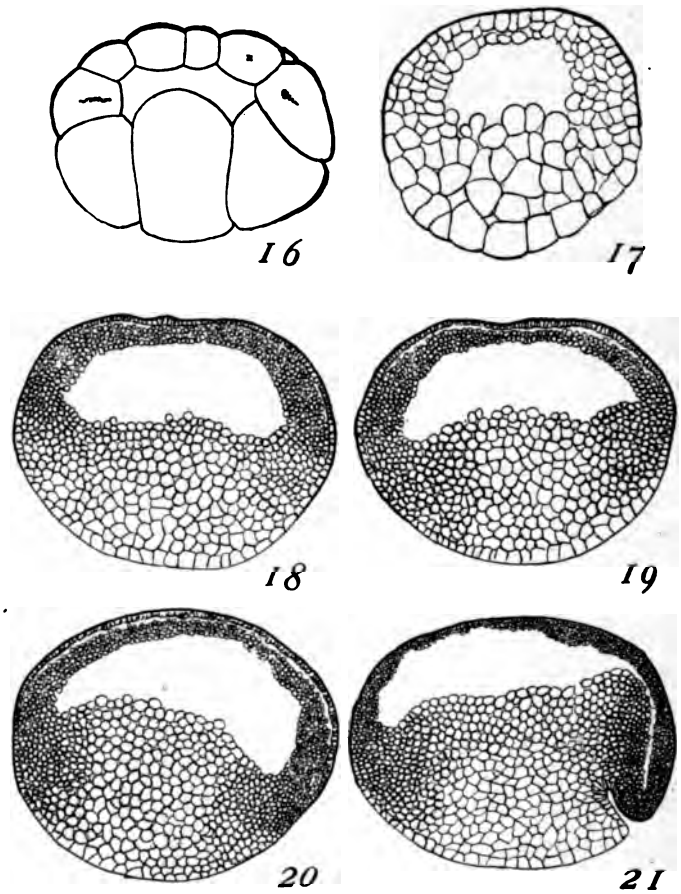
drawn to larger scale than the preceding. Since the cells are dividing during this time without the number of layers in the roof of the blastocœl becoming correspondingly greater — in fact they are fewer in the gastrulation stages — this must mean that as the cells increase in number they push out towards the sides, and increase the material of the embryo-ring, which is, *pari passu*, pushing below the equator of the egg.

Rana sylvatica. — Owing to the looseness of the cells of the roof of the segmentation cavity in this species — whether representing a real condition or due to the effects of the preserving reagents I do not know — the preparations show less well the thinning out of the roof of the blastocœl. The first stage figured, Fig. 16, is a section through a 32-cell stage. The inwandering of the upper cells has not yet been accomplished, the segmentation cavity is well developed, and its roof is thick. The upper ends of the four lower cells of the eight-cell stage are, at this time, cut off by the fifth divisions, that usually lies obliquely. In the figure these cells are represented by the two large cells at the sides. They contribute, I think, much of the material that forms the "embryo."

The next figure, Fig. 17, shows a later stage, cut somewhat obliquely. The blastocœl is large, and its roof thinner, but the sides are as thick as before, or nearly so, as shown by superposing the 32-cell stage upon this one. The next two figures, Figs. 18–19, show a broadening of the blastocœl, and thinning of its roof, accompanied by a downgrowth of the small cells around its sides. The next figure, Fig. 20, shows the downgrowth carried further, and the yolk floor correspondingly lifted up. The last figure, Fig. 21, is through the dorsal lip of the blastopore, at the time when the invagination has just appeared. The roof of the segmentation cavity is thinner and continuous at the anterior end with the ectoderm in front of the dorsal lip. This ectoderm is now separated from the cells inside; except in the dorsal lip itself, where ectoderm and mesoderm completely coalesce.[1] A tongue of small cells, running upwards from the blastopore beneath the ectoderm, represents the mesoderm. This mass of cells along with the yolk-cells

[1] Possibly ectoderm cells are added along the middle line to the sheet of mesoderm and give rise to the notochord.

with which it is continuous further inwards, projects upwards at the anterior end of the egg into the blastocœl. On the opposite side of the section, the downgrowth is less developed, and the yolk-floor less elevated.

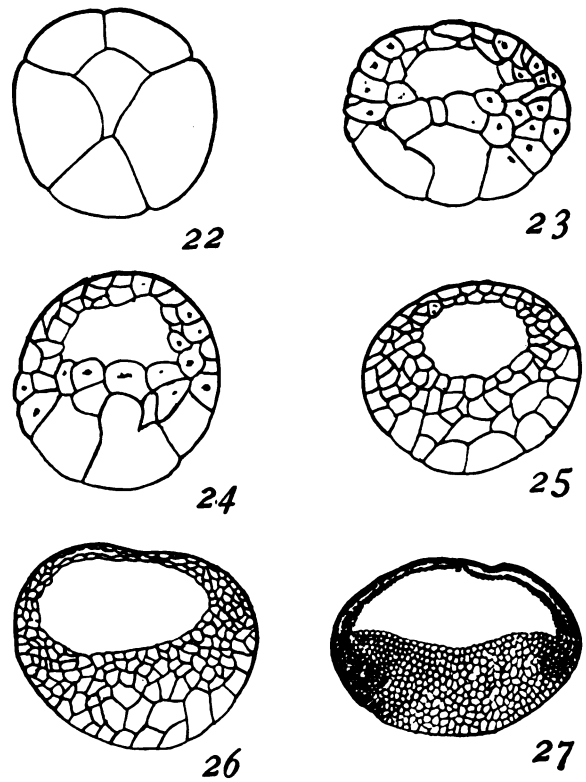


FIGS. 16–21. Segmentation and gastrulation stage (Fig. 21) of *Rana sylvatica*.

The general conditions in this species are the same, in all essential respects, as in the last.

Bufo lentiginosus. — The toad, although belonging to another family of the Anura, follows very closely the same method of early development as the frog. In several respects it shows with great clearness some of the early changes that I have described

in the frog's egg. A section through an eight-cell stage is shown in Fig. 22. The thickness of the roof and the sides of the blastocœl, and the extension of the blastocœl far down into the lower hemisphere is very noticeable. In the next stage, Fig. 23, the roof is thinner, and the segmentation cavity extends out more horizontally. The same changes are seen in the next figure, Fig. 24, which is an egg in the same stage of development, but compressed, in cutting, in a plane at right angles to that of the last figure. An older stage is seen in the next figure,



FIGS. 22–27. Segmentation and gastrulation (Fig. 27) stages of *Bufo lentiginosus*.

Fig. 25, where the conditions are much the same. The small embryo-forming cells still lie above the equator. In the next stage, Fig. 26, the segmentation cavity is greatly enlarged, its roof is much thinner, and now the embryo-forming cells lie at

about the equator of the egg. It is evident, when this stage is compared with the last one, that an extensive shifting of the cells must have taken place. This is strikingly shown by superposing the outline of Fig. 22 upon that of Fig. 26. The next stage, Fig. 27, shows important changes to have taken place in the small cells around the sides of the segmentation cavity. These have now pushed below the equator of the egg, and the wall in this region has correspondingly diminished in thickness. This last section is through an embryo that has just reached the gastrulation stage, and although the section passes vertically through the greater diameter of the egg, the blastopore lip lies slightly to one side, and opens in neighboring sections that are not quite so large. The mesoderm cells, containing more pigment than the endoderm cells, form a distinct tongue extending upwards from the level of the blastopore to the floor of the archenteron. The general appearance of the section suggests strongly that the innermost cells have remained stationary, or have even pushed upwards a little, accompanying the general upward movement of the floor of the archenteron.

The later stages of the gastrulation-process in the toad, I have not studied. King has recently given a series of figures showing the later development. They show that the yolk-mass is thrown high up into the blastocoel, especially at the anterior end, so that the anterior part of the archenteron comes to lie higher up than the original floor of the archenteron. It is not improbable that the embryo of the toad lies higher up on the anterior side of the egg than does the embryo of the frog, although the difference is only one of degree.

During the period of closure of the blastopore, the large blastocœl becomes filled by the yolk and the drawing inwards of the yolk brings the dorsal and ventral lips together without, in a sense, their actually growing *over* the lower hemisphere.

Conclusions.

The large number of cells in the frog's egg at the time when the embryo appears makes it impossible to trace the cell-lineage after five or six divisions, so that we must be content with less refined methods in locating the embryo-forming materials.

Whether the inwandering, or inpulling of some of the cells of the upper hemisphere is only due to the shifting of the cells as they divide, so that the pressure relations are better adjusted, or whether there is a further meaning to be attached to this process cannot be stated. One might be tempted to assign to the cells the function of producing the mesoderm, and the later pushing outwards at the sides of much of the material derived from these cells into a position where the mesoderm appears might be made to give color to this interpretation. However this may be, the experiment of removing the upper four cells shows that some, at least, of the mesoderm comes from other parts of the egg. Whether the same amount forms under these circumstances is too difficult to determine. In the later stages of gastrulation, and at the time also when the neural plate is forming, the mesoderm on the ventral surface increases at the expense of the yolk-cells. These are the yolk-cells that form the tongue of cells that pushes upward into the blastocœl.

In my last paper dealing with the gastrulation of the frog's egg, I discussed the "mechanics" of the downward movement of the material that forms the embryonic ring. Certain points that bear on this question have been noted in studying the three species here described, and may be briefly mentioned. There seems to be a good deal of variation in the extent to which the blastocœl enlarges, not only in different species, but in the same species when eggs from different bunches are compared, and even to a slight extent in eggs from the same bunch. The segmentation cavity appears to be formed as a result of the secretion of material from the surrounding cells, as shown by its occurrence in eggs developing out of water. The egg as a whole increases in size as the segmentation cavity grows larger, indicating that the water absorbed from outside more than compensates for the amount of fluid secreted into the central space.

The thinning of the roof of the segmentation cavity takes place at the time of enlargement of the segmentation cavity and it may appear that this enlargement is the cause of the downward movement of the material; but that this is not the correct interpretation of the mechanics of the process is shown by the following experiment. The top of the segmentation cavity was

opened and partly removed, by means of a needle, at different stages in the cleavage process. Nevertheless, the materials that form the embryonic ring continued to push down at the sides of the egg and a normal embryo was produced. The experiment is open to the obvious criticism that after the removal of the roof, or a part of it, the opening soon closes again; but if the downgrowth were really due to pressure of the blastocœl fluid or even to the pushing of the cells of the roof on each other, the disorganization of the process, that would probably follow, when a part of the roof is removed, must be so great, one would think, that it is unlikely the downgrowth could subsequently take place normally. Still the experiment is inconclusive and does not settle the question.

The two most conspicuous changes that take place during the segmentation and gastrulation stages are the development of the enormous segmentation cavity and its disappearance during gastrulation. We may look upon the *purpose* of the segmentation cavity as a space into which the lower cells may push when the upper cells pass over them. While the presence of the segmentation cavity may facilitate this process, we gain no insight into the origin of the cavity or of its disappearance by a consideration of its purpose. Its formation seems to be due largely, as I have already said, to a secretion of fluid from the surrounding cells; its disappearance is more difficult to explain. The question resolves itself into these alternatives — is the blastocœl fluid absorbed or is it forced out of the egg in the later stages of gastrulation? Since the egg does not decrease in volume or very little, as the yolk surrounds and finally obliterates the segmentation cavity, the fluid would seem to be absorbed. It is true, we might assume that the fluid is squeezed out of the egg and water from the outside absorbed at the same rate, but this assumption only complicates the question, and in the end amounts to the same thing. Admitting that the fluid is absorbed, can this explain the inward migration of the yolk. I think not, because in the first place the process of ingrowth takes place around the sides of the blastocœl cavity and around the lips of the blastopore and not throughout the entire floor of the segmentation cavity, and in the second place there is nothing in the changes that take place

to indicate that they are due only to the absorption of the blastocœl fluid, since the cells that undergo the changes in question do not appreciably grow larger than the others. On the contrary the gastrulation seems to be due to the change in shape and migration of certain cells, and the absorption of the fluid from the blastocœl appears to be no more than the ordinary process of water absorption that takes place throughout the whole period of development.

The evidence that we have at present seems to indicate that the process of gastrulation is due to the activities of the cells themselves, *i. e.*, the "mechanics" of the process can only be explained by an appeal to the response of the cells to certain stimuli. The actual changes that we observe involve a change in shape of certain cells, and our problem resolves itself into determining what stimulus leads to this change in shape, and what physical process is involved in the change. I have pointed out that it seems to me probable that the stimulus is derived from the mutual pressures of the cells, and the change of shape is due to contraction processes in the cells (that lead to their change in shape) that are akin to the same contraction shown by a protozoön, or, in a higher form, by muscle cells. The different behavior of the cells in different parts of the egg must be ascribed to their difference in materials that are derived from the different parts of the egg. After each cell-division, itself apparently a process of local contraction, there must be a rearrangement of the pressure relations in the different parts of the egg.

The formative factors of development can be reduced, from this point of view, to the two generally recognized properties of living matter, irritability and contractility. The stimulus that arouses the irritability is the pressure relation of the cells; the time at which each cell is effected by this stimulus will depend on its material composition, and it responds by its contraction. If this interpretation of the formative changes is correct it refers the process of development to two physiological properties of living matter, and not directly to other known physical properties of organic matter. What the physical bases of irritability and of contractility are remains for the future to decide. They may be

simply complexes of known physical factors, or they may be physical properties of organic matter that are not met with in inorganic compounds. Whatever their ultimate nature it would be a distinct gain if we could prove that most of the phenomena of early development, the so-called formative changes, are due to these two properties of living substance.

A NOTE ON THE SUSCEPTIBILITY OF SEGMENTING ARBACIA AND ASTERIAS EGGS TO CYANIDES.[1]

A. P. MATHEWS.

The discovery by Lyon[2] that after fertilization sea-urchin eggs show definite periods of resistance and susceptibility to the action of cyanides is of such general interest that it is important to establish the exact period at which eggs are most sensitive and to discover whether other eggs also show similar periods of susceptibility. Lyon found that the eggs were most easily killed about the time of the first cleavage. He was in doubt as to the exact period, but thought it came just after the cleavage, whereas immediately preceding cleavage the egg was very immune. He made no sections and was not entirely certain with just what processes in the egg these periods of immunity and susceptibility coincided. Spaulding[3] tried similar experiments using ether and acids. The ether especially gave a very sharp result and showed great susceptibility immediately *preceding* segmentation. The acid, as might be anticipated from the various actions it possesses, gave a far more complicated series of phenomena. It seemed desirable to ascertain exactly the period of susceptibility to the cyanides and to discover if possible what series of changes within the egg corresponded with the periods of susceptibility and immunity. A study was accordingly made of the living eggs of *Arbacia* and *Asterias* and I have reëxamined a series of sections of *Arbacia* eggs made several years ago, in which the eggs were preserved in sublimate-acetic at definite intervals after fertilization. I also endeavored to repeat Lyon's observations on *Arbacia* using *Asterias Forbesii* (?) eggs. Some very fine material was gathered about the middle of September at Woods Holl.

[1] Laboratory of Biochemistry, University of Chicago, and the Marine Biological Laboratory, Woods Holl.

[2] Lyon, *American Journal of Physiology*, VII., 1902, p. 56; XI., 1903, p. 52.

[3] Spaulding, BIOLOGICAL BULLETIN, VI., 1904, p. 224.

The sections, the living material and the observations on *Asterias* indicate that the period of great susceptibility is *immediately before and during segmentation* and that just after segmentation great resistance prevails.

In the following table I have placed in parallel columns one of Lyon's results, and the phenomena as shown in sections of the eggs. There is one objection to comparing different series of eggs in that the temperature may not have been the same in the different experiments, and the periods will not exactly coincide. However the eggs of *Arbacia* develop very uniformly and nearly always the first cleavage comes in between fifty and sixty-five minutes after fertilization.

TABLE I.

Minutes After Fertilization.	Susceptibility to Cyanide	Phenomena as Shown in Sections and Living Material
0	Slight.	Egg at rest.
0–10	Slight increase in susceptibility.	Sperm penetrating egg. Aster very small. Meets egg nucleus in 8–12 minutes.
10–25	First period of susceptibility.	Fusion of nuclei. Great growth of aster. Caterpillar stage. Rays throughout cell.
30–48	Progressive increase in immunity. At end most immune.	Pause stage and retrogression of aster. Large rays fade out. Enormous growth of nucleus.
48–60	Second period of susceptibility.	Nuclear wall fades about 45–50 minutes after fertilization. Tremendous growth of asters follows At 60 minutes chromosomes beginning to separate.
60–65	Susceptible.	Segmentation completed.
65–70	Immune.	Retrogression of asters.

It will be seen from Lyon's experiments that as a rule his period of susceptibility came about fifty-five minutes after fertilization or even five minutes earlier. This period is certainly just before segmentation.

It is clear from this table that the period of great susceptibility coincides with the development of the asters; and the period of greatest immunity coincides with the retrogression of the asters and the development of the nucleus. Thus the first decrease in immunity coincides with the time when the sperm aster is developing. This reaches its maximum about 20–25 minutes after

fertilization. There then ensues a period of retrogression of the aster, the astral radiations fade out, and the nucleus grows enormously. This is the immune period. At the end of that time the nuclear membrane disappears very rapidly and a great growth of the aster takes place, leading up to the first division. This is the second susceptible period. Following segmentation, the nucleus reforms. The asters again decrease in size, and a second period of resistance occurs. This is, however, short, as the second division comes very quickly.

I have attempted to confirm these observations by studying the living *Asterias* egg. I got a very fine lot of *Asterias* eggs, nearly the whole starting maturating within five minutes after shedding. They were fertilized very soon after the germinal vesicle had begun to disappear and transferred at ten-minute intervals (at five-minute intervals during the extrusion of the polar globules and segmentation) to *m*/100, *m*/50 and *m*/25 sodium cyanide solutions. After remaining in the cyanide solution for periods of one, two and three hours they were transferred to fresh sea-water, which was repeatedly changed, and were then left to develop.

The results were very unsatisfactory. No sharp periods of susceptibility could be discovered in which the majority of the eggs were killed in the two-cell stage as in *Arbacia*. This failure could not be attributed, I think, to lack of uniformity in the development of the egg, since they segmented with marked uniformity. In practically all cases development proceeded after removal of the cyanide, although there were in some cases marked differences in the appearance of the embryos in lots introduced into the cyanide at different times. Those eggs put in the cyanide immediately after segmentation formed swimming gastrulæ which were fairly normal. Eggs introduced into the cyanide immediately before segmentation had the larger number of dwarf and irregular blastulæ and disintegrating eggs. Frequently the eggs after removal from the cyanide did not segment for several hours and then broke at once into a mass of spheres, some of which died, while others formed the embryo.

The resistance of the eggs was remarkable. One series was left for three hours in *m*/25 cyanide, and the great majority of the

eggs in every lot formed swimming embryos when restored to sea-water.

While the results were thus not sharp and decisive, as in *Arbacia*, and the resistance of the egg to the cyanide very much greater, the experiments were generally harmonious in showing more abnormal embryos in the period just preceding segmentation. In the one exception there were no clear differences between the eggs introduced into the cyanide at different periods.

Why the *Arbacia* eggs are more susceptible when the asters are developing is of course entirely obscure. At this period it is probable, from unpublished observations, that an oxidase escapes from the nucleus and exerts its action upon the cytoplasm. It is possible, also, that at this period the reducing substance in the centriole becomes more active. By an increase in activity is meant an increase in combining power. It may be, therefore, either that the cyanide prevents the oxidase from exerting a necessary action, or it combines with the active centriole and destroys its action, whereas it does not combine with the inactive centriole substance. Further investigation is necessary to decide whether either of these suggestions is the correct explanation. The great resistance of the eggs of *Asterias Forbesii* to the cyanide cannot be explained without further experiment.

A NOTE ON THE STRUCTURE OF THE LIVING PROTOPLASM OF ECHINODERM EGGS.[1]

A. P. MATHEWS.

From a study of the living protoplasm of several different echinoderm eggs Wilson[2] came to the conclusion that the structure was alveolar, thus confirming Bütschli. During the past summer in connection with the study of the chemical basis of mitosis I had occasion to repeat his observations and have been led to a somewhat different conclusion.

I examined the living eggs of *Arbacia punctulata* and *Asterias Forbesii* under slight compression, using Leitz $\frac{1}{12}$ oil immersion and ocular 4.

In my opinion the phenomena one observes in the living fertilized egg show that the structure of these eggs is not correctly represented by the term alveolar. The protoplasm seemed to me to consist of a perfectly clear, homogeneous matrix in which no trace of structure could be seen, but which contained a vast number of small granules. These granules differ somewhat in size, those near the periphery of the egg being somewhat larger than at the center, and throughout the egg, but particularly near the surface, a great number of very minute granules or microsomes may be seen.[3]

The description of the protoplasm agrees with that given by Wilson except that he regards these granules as alveoli filled with fluid. That they are more correctly regarded as granules in a homogeneous matrix may be shown in a variety of ways.

For example, the granules may be isolated in sea-water if the egg is first treated with sodium sulphate or sodium iodide, $\frac{5}{8}N$, or even in some cases with neutral red. The granules after such

[1] From the Marine Biological Laboratory, Woods Holl.

[2] Wilson, *Journal of Morphology*, XV., 1899, p. 6.

[3] These observations confirm those of F. R. Lillie on the *Chætopterus* egg (Lillie: Personal communication) and of E. P. Lyon on *Arbacia* (see Lyon: Amer. Jour. of Physiol., 1906, vi).

treatment do not swell and disappear as they ordinarily do on crushing the egg, but persist as granules and may be separated from their surrounding matrix. They then acquire a very active Brownian movement and float off as granules.

That they exist as granules in the egg is shown also by their behavior during mitosis and in the centrifuge. In *Arbacia*, the granules are some of them pigmented. During mitosis, these granules do not remain evenly distributed over the surface of the egg, but collect along the furrow of segmentation. Also when the astral centers are formed the granules either move away from the neighborhood of the asters, as has been described for other eggs, or else they are dissolved in these areas. At any rate near the asters and spindles only the clear protoplasm without granules is to be found.

Furthermore, when the large astral radiations extending to the surface of the egg are formed, it may be seen that these radiations consist of the clear homogeneous matrix. The granules are arranged in rows between the broad clear rays. When oxygen is taken away the granules move in again where the rays were.

Further evidence is obtained if the eggs are centrifugalized in the method discovered by Lyon at Woods Holl, this summer. By centrifugalization the granules are separated into zones of granules, as has been described by Lyon and Lillie and the clear protoplasm forms one zone by itself.

The clear protoplasm which forms the matrix in which the granules are, is oftentimes extremely viscid. This is shown in several ways. The granules possess, as long as the egg remains normal, no Brownian movement, showing that they must be in a viscid matrix. It is only when the egg is partially liquefied that they acquire Brownian movement. The viscidity is also shown by observations on living star-fish eggs partially deprived of oxygen. Long viscid strands can then be seen extending between the blastomeres, with here and there granules embedded in them.[1] If these eggs are beaten with a rod, it is frequently possible to draw the protoplasm out in long clear threads.

[1] See Mrs. Andrews : Journal of Morphology, 1896, xii, p. 367.

I do not mean to imply that these granules are hard insoluble granules because many of them, as Wilson has pointed out, appear more fluid than solid, but I mean that the actual structure of protoplasm is more clearly and accurately represented as a clear homogeneous viscid matrix, in which oil globules and all sorts and sizes of granules are embedded than as an alveolar structure.[1] An alveolus is a hole in a matrix. It is not the hole, but the matrix, which in my opinion is the important part of protoplasm.

If one seeks for a substance most nearly resembling protoplasm in structure, raspberry jam, were it colorless, would fairly accurately represent what one sees — a clear sticky matrix in which are a multitude of seeds.

In none of the eggs while living could I see any trace of spindle fibers or of fibers at all like those seen in sections. When the egg is fixed, the proteid colloids which in solution constitute the larger part of the clear viscid matrix, are precipitated in a granular form. In other words, the clear matrix appears to represent a colloidal solution in which our fixing agents throw down a precipitate. The appearance of rays seen in fixed preparations is quite different from appearances in the living egg. In the living egg the clear protoplasm sends off radiating broad strands toward the periphery of the cell. In fixed preparations the rays appear a great deal stiffer and much finer, not so broad as the clear bands although they occupy the position of the living radiations.

These observations indicate very clearly to my mind that the protoplasm of these eggs is essentially a viscid colloidal solution something like gum arabic in viscidity and embedded in it are precipitated or undissolved granules. It may be regarded as a partially precipitated, colloidal solution and the ease with which the granules may be made to dissolve in the clear matrix, indicates, I think, that the amount of precipitate and number of these granules must be constantly changing. Protoplasm appears to be in fact a two-phase colloidal system, undergoing spontaneous chemical change, and surrounded by a semi-permeable membrane.

The changes the granules undergo in the living egg when the latter is compressed or burst are extremely difficult to follow and

[1] From *Asterias* eggs large quantities of an oil may be separated.

interpret. The granules fade out or seem to dissolve in whole or in part in the viscid matrix. This matrix with the dissolved granules, when in contact with water, rounds itself up as if oily and the crushed protoplasm at first as it flows from the egg breaks into a mass of spheres consisting of viscid matter with dissolved granules, and these spheres then ultimately dissolve almost completely in the water.

The speed of solution of the granules is extraordinary. In some cases where the egg was too much compressed by the coverglass and lens, in a few seconds, almost all the granules in the egg disappeared.

These granules all stain in the basic dyes *intra vitam*, and never in acid. I found, however, that if eggs which have died in the ovary are brought into the dyes, all the dead eggs stain in the acid dyes; all the living in the basic. A change in staining reaction, probably coincident with a change in reaction from alkaline to acid, thus occurs at death.

If eggs in which the granules are stained with any basic dye be crushed, it will be observed that the color disappears when the granule dissolves. What becomes of the color is a puzzle. It does not stain the matrix nor appear to color the sea-water. It may be that it is destroyed by the chemical change taking place.

The extreme sensitiveness of the granules, and the abruptness with which the change in appearance of the protoplasm occurs, suggests that possibly this is a physiological process to be correlated with some of the vital phenomena, *i. e.*, contractility.

Dr. R. G. Davis examined, at my suggestion, the chemical composition of the star-fish egg. He succeeded in isolating from the eggs a large amount of an oil, fluid at ordinary temperatures. The chemical nature of this oil has not been determined. I think it possible that many of these granules represent that oil. The oil is very likely partially saponified and contains a small amount of fatty acid. By the dissociation of this acid the drop or oil globule becomes electro-negative. It is in this way the globule acquires the power of staining with basic dyes, since the insoluble soap is formed. The quick disappearance of the granules when compressed, giving one the impression that they are composed of a fluid which mixes with the viscid matrix, and

their quick fusion under the influence of ether, also bear out this interpretation. It is not impossible that ordinarily the granules or oil drops are in combination of a peculiar kind with basic proteids of the protoplasm, the whole forming a highly unstable and complex compound. The chemical composition of these granules is being studied more closely.

PRELIMINARY REPORT ON THE EMBRYOLOGY OF CRYPTOBRANCHUS ALLEGHENIENSIS.[1]

BERTRAM G. SMITH.

Although the eggs of the hellbender, *Cryptobranchus* (*Menopoma*) *alleghenicnsis*, have been eagerly searched for by embryologists, a few unfertilized eggs only have hitherto been obtained. Aside from a brief description of an embryo in an advanced stage by McGregor ('96), there is no record of any observations on the development. The manner in which the eggs are fertilized has not been described.

This gap in our knowledge of comparative embryology is serious, particularly as very little is known about the development of the two other members of the family[2] Amphiumidæ: the giant salamander of Japan, *Cryptobranchus japonicus* (*Megalobatrachus maximus* Schlegel), and the American *Amphiuma means*.

During the early part of September, 1905, in northwestern Pennsylvania, I was so fortunate as to obtain fertilized eggs of *Cryptobranchus alleghenicnsis* in abundance, both from specimens in captivity and from the natural habitat of the animal.

I. The Sexual Elements.

A. *The Egg.* — The egg proper is perfectly spherical when fresh, about the size of a large pea, and bright yellow in color — a rather deep yellow at the lower pole, grading to a pale yellow at the upper. There is no black pigment such as is found in the eggs of most amphibians. A very thin transparent membrane, the vitelline membrane, quite inconspicuous in fresh material, closely invests the egg. Each egg, with its vitelline membrane, floats in a clear fluid within a hollow sphere or capsule about the size of a large grape, formed by the thick gelatinous outer

[1] Contributions from the Zoölogical Laboratory of the University of Michigan, No. 105.

[2] Gadow's classification is followed ('01).

envelope, the secretion of the oviduct. This outer egg envelope is produced at opposite poles of the capsule to form a slender cord connecting the eggs in a string (Fig. 1). The envelope is

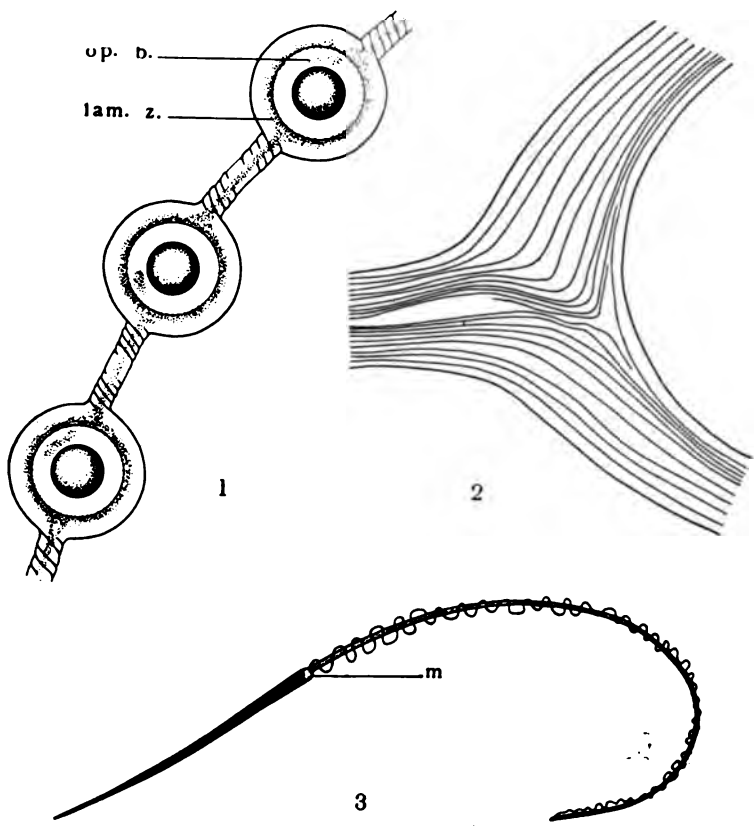


FIG. 1. Eggs and egg envelopes of *Cryptobranchus*, natural size. *op.b.*, opaque body; *lam.z.*, lamellar zone of envelope.

FIG. 2. Optical section (longitudinal) through lamellar zone of envelope in region of junction of egg capsule with connecting cord. Magnified about thirteen diameters.

FIG. 3. Spermatozoön, magnified about five hundred diameters. *m.*, middle piece.

perfectly transparent when fresh, except that wherever viewed tangentially its inner layers have a milky appearance, represented by the shaded zone in Fig. 1, and due to a fine lamellar structure sketched in optical section, with a magnification of about thirteen diameters, in Fig. 2. The misty appearance is

caused by the diffusion of light passing through these concentric layers tangential to their inner surfaces. The core or axis of the connecting cord also has the same milky appearance, due to a continuation of the lamellar structure.

The outer surface of the capsule and cord is, to ordinary observation, perfectly smooth and regularly rounded when the material is in fresh condition. But a careful examination, especially with the aid of a lens, reveals a delicate longitudinal striation due to fine parallel grooves or scratches separated by nearly equal intervals. This structure is too minute to be represented in Fig. 1. In some portions of the string the striæ have a slightly spiral direction about the axis of the cord and capsule. The striæ appear as they would if made by the scratching of the surface of the envelope against the teeth of a fine comb; perhaps they are caused by friction of the envelope against papillæ of some sort in the lower portion of the oviduct or in the cloaca, while the gelatinous material is yet soft.

After the eggs have been in water for several days, or after preservation in formalin or alcohol, the outer layer of the egg envelope becomes cast into conspicuous wavy folds or wrinkles of a different nature from the striæ noted above. These folds appear first at the ends of the connecting cord adjacent to the egg capsule and have here a strongly marked spiral arrangement, suggestive of the chalazæ of the hen's egg (see Fig. 1); later the folding may extend over the capsule and the middle of the cord, but seldom with so regular a spiral arrangement in these regions. Sometimes the spiral is continuous from one capsule to another. As a rule the spiral is constant in the direction in which it extends about the axis in all portions of the cord and capsule.

The inner layer of the lamellar core of the cord in some cases exhibits a marked twisted or spiral arrangement like that of the inner portion of the cord connecting the eggs of *Ichthyophis* as described by the Sarasins ('87–'93).

The following measurements were taken:

(1)	Diameter of egg proper,	7 mm.
(2)	" " " with envelopes,	18 "
(3)	" " connecting cord,	5 "
(4)	Distance from one egg to another, measured from center to center,	3 cm.

These figures represent the average of several measurements, the range of variation of the first dimension being small, of the others considerable, in eggs from the same parent. The egg proper ordinarily sinks to the bottom of the fluid which surrounds it, so that when viewed from above it is magnified by the lens-shaped capsule so as to appear considerably larger than is indicated by direct measurement.

Occasional strings of egg capsules are found without any eggs enclosed. These empty egg envelopes have the same structure as the others, and usually occur as a portion of a string of capsules the remainder of which contains eggs. A few of the empty egg capsules are double, formed by the union of two capsules without a connecting cord; in such cases the cavities of the two capsules are separated only by a very thin gelatinous septum.

In fertilized eggs in an early stage of development, preserved in formalin without removal from their envelopes, I found floating in the liquid between the egg and its envelope a fairly large irregular and slightly opaque mass, in appearance like a faint white cloud (see Fig. 1). This mass had not been noticed in the capsule of the living egg, probably because it was then transparent. Under the microscope it was found to consist of a clear gelatinous matrix in which were embedded numerous large round cells each with a very large nucleus and often with what appeared to be a large vacuole. By the use of a dissecting lens the individual cells could be seen while still within the envelope. In some egg capsules they occur diffused throughout the liquid of the cavity, not aggregated in a mass to form an opaque body. Numerous spermatozoa are present in the opaque body, but they also occur floating in the surrounding liquid and embedded in the egg capsule. Whether the opaque body is present in unfertilized eggs I have not at present the material to determine. The unfertilized eggs of *Cryptobranchus allegheniensis* have been briefly described by Reese ('04) but without mention of any such feature as the opaque body described above. Both opaque body and spermatozoa regularly occur in egg capsules that do not contain eggs. The origin and function of the cells of the opaque body are at present problematical.

The eggs of *Cryptobranchus allegheniensis* bear a rather close resemblance to those of *C. japonicus* as described by Sasaki ('87), Kerbert ('04), Ishikawa ('04) and de Bussy ('05). Other amphibians whose eggs are fastened together like a string of beads are *Amphiuma* (Hay, '88 and '90), *Desmognathus*, *Ichthyophis*, *Hypogeophis* (Brauer, '97), and *Alytes*. The eggs of *Bufo* are arranged in strings but without the marked constriction of the envelope between the eggs characteristic of the other forms mentioned.

B. *The Sperm.* — The spermatozoön (Fig. 3) is of considerable size, about 225 μ long, but with an unusually small middle-piece. The head of the spermatozoön, excepting the tip or acrosome, stains deeply with Conklin's modification of Delafield's hæmotoxylin. The head is very long and slender, tapering gradually to the extremely fine-pointed acrosome, enabling the spermatozoön to pierce easily through the vitelline membrane. The tail-piece is provided with an undulating membrane, bordered with a convoluted filament.

The spermatozoön of *Cryptobranchus allegheniensis* resembles that of *C. japonicus* as figured by Ishikawa ('04). It is also quite similar to the spermatozoön of *Amphiuma* (McGregor, '99), but the latter has a larger middle-piece and a slightly barbed acrosome.

II. Breeding Habits.

A. *Method of Fertilization.* — A newly-captured female was isolated in an aquarium not previously occupied by any other specimen. A large number of eggs were laid, which contained spermatozoa within the egg capsule; moreover the subsequent development of these eggs proved them to have been fertilized. Hence there can be no doubt that in *Cryptobranchus alleghenicnsis*, as in all other Urodeles so far as known, fertilization, unlike that of the *Anura*, is internal. The mode of transference of the male element to the cloaca of the female has not been ascertained.

Nothing conclusive has been established concerning the method of fertilization of the nearest relative of *Cryptobranchus alleghenicnsis*, the giant salamander of Japan. In the case of *Amphiuma*, Davison ('94 and '95) believed a transference of spermatozoa

from male to female to take place by means of an apposition of the lips of the two cloacæ, basing this belief on what appears to me insufficient evidence; such a process is moreover not in harmony with known methods of fertilization among the Urodeles, in which fertilization is effected by means of spermatophores and without direct cloacal contact (Jordan, '91 and '93). Internal fertilization is said to occur in the Gymnophiona, *Ichthyophis* and *Hypogeophis*, as well as in the Urodeles.

B. *Breeding Season.* — Fertilized eggs were laid by a specimen in captivity on September 6, and another spawning by the same female took place during the night of September 7–8. Eggs in the first cleavage stage were found in the natural habitat of the animal on September 7, and another spawning of eggs in an advanced stage of segmentation was found in the same habitat on September 8.

Townsend ('82) records the laying of some eggs by a specimen in captivity during the month of August. McGregor ('99) states that the eggs are deposited in August and September.

No direct observations as to the time when the females are fertilized were obtained; but male specimens killed during the early part of September were found to have the vasa deferentia distended by a large quantity of seminal fluid containing an abundance of ripe spermatozoa. According to McGregor ('99) "the sexual union must occur very near or at the time of egg-laying, for the female is devoid of spermathecæ, and the spermatozoa do not ripen until late in August."

Eggs of *C. japonicus* were found by Sasaki ('87) in August. Kerbert ('04) records the spawning of a specimen in captivity during the night of September 18, 1902, and again on September 19, 1903. DeBussy ('05) reports that eggs were laid by Kerbert's specimen during September 14, 1904, and several days following. According to Ishikawa ('04) the eggs are laid principally during the latter half of August, but also in September. Hence the breeding season coincides with that of *C. alleghenicnsis.*

Eggs of *Amphiuma* in an advanced stage of development were found by Hay ('88 and '90) on September 1. Davison ('94 and '95) states that the eggs are deposited in August or September, but without giving the data upon which this statement is based.

He found a viscid substance containing spermatozoa exuding from the vent of a male specimen in May (April, according to his earlier paper), and concluded that this month is the natural season for fertilization.

The occurrence of the breeding season of *Cryptobranchus* in the fall is in marked contrast to the habits of nearly all other Urodeles, since they lay their eggs in the spring. While the significance of this unusual breeding season is not readily apparent, it is, in the case of *C. alleghenicnsis*, in at least one respect adaptive. The animal is an inhabitant of streams that during spring and early summer are subject to frequent and destructive freshets, which would probably be disastrous to the development of eggs like those of *Cryptobranchus*. During late summer and fall the streams are shallow and the water comparatively quiet; floods are of rare occurrence. These factors do not affect the other amphibians of the region in the same way, since they are inhabitants of ponds not seriously disturbed by floods, and on account of the more abundant rainfall better adapted for breeding-grounds in the spring than later in the year. There is every reason to believe that these climatic conditions have been of long duration. Probably the same conditions prevail with regard to *C. japonicus*, which is an inhabitant of mountain streams similar to those in which *C. alleghenicnsis* occurs. In this connection Professor Jacob Reighard informs me that the increase of spring freshets in Michigan during recent years, aided no doubt by other effects of lumbering operations, has nearly caused the extinction of the grayling, a fish that breeds in the spring, and was formerly abundant; the trout, which breeds in the fall, now thrives in the same streams. These facts indicate the selective value of the factors mentioned, and support the view taken with regard to *Cryptobranchus*.

C. *Breeding Habitat.* — Eggs of *Cryptobranchus* were found in shallow water in what had once been the main channel of a large stream, but through which now only a portion of the water, separated from the main channel by an island, flows. This old river channel is extremely rocky, with a considerable incline, so that the shallow water alternately forms pools and rapids. Judging from the number of specimens seen, the locality is a

favorite haunt of *Cryptobranchus*. Adult hellbenders were seen in other portions of the stream in deeper water, but in no other situations were eggs found.

The locality described above bears a strong resemblance to the habitat of *C. japonicus* as illustrated by Ishikawa ('04).

D. *Habits of Oviposition.* — Two separate spawnings of eggs were found in the natural habitat of the animal, besides eggs laid on two different occasions by a specimen in captivity. On account of differences in the method of disposal of the eggs, these spawnings are best described separately.

The eggs found on September 7 were lying in gently flowing water about 2–4 inches deep, on a gravelly and stony bottom, within a space about 6 feet in diameter nearly enclosed by some large rocks which projected a foot or more out of the water. The eggs were arranged in long festoon-like strings, scattered over an area of about 2 x 5 feet. In a few places the eggs were grouped in masses, but these masses might readily be resolved into strings. All the eggs present were included in a very few strings; one string contained 27 eggs. Evidently the eggs had not been disturbed since being laid. One hundred and ten eggs were counted in full view; but some other eggs had sunk down into crevices between and beneath stones; these brought the entire number up to 135. The eggs lay for the most part in direct sunshine, and the shallow running water furnished them with abundant aëration. No adult hellbenders were seen in the immediate vicinity.

The eggs were conspicuous because of their size and number, and were really beautiful objects, on account of the regularity of their form, the festoon-like manner of their arrangement on the pebbly bottom, the bright yellow of the yolk, and the perfect transparency of their gelatinous envelopes except where bordered with a delicate misty gray.

On September 8 another spawning of eggs, perhaps 50 in number, was found not far from the spot where eggs were discovered on September 7. The eggs were in water 3–5 inches deep, on the down-stream side of a rock, and most of them in a shallow cavity about 16 inches wide and extending back about 8 inches under the rock. From the appearance of the surround-

ing and underlying gravel this hollow looked as if it had been dug out by some animal. The eggs had been disturbed and scattered considerably; the strings were short, as if they had been much broken up, and many separated eggs were found. The eggs were thickly covered with silt. Both the envelopes and the contained eggs were, as a rule, slightly larger than those previously found. No adults were seen in the vicinity.

The eggs obtained on September 6 from a specimen in captivity were found rolled and tangled together in such an intricate manner that they seemed to occur in clusters or masses rather than in strings. The number was estimated at about 80, nearly all in one oblong mass. The solitary female did not appear to care for the eggs in any way.

On September 8 about 300 more eggs were laid by the same female specimen in captivity. The strings of eggs were aggregated in one large mass, but they were not so much tangled as in the case of those laid previously. There were present at the time in the same aquarium three male specimens, but so far as was observed, none of the adults paid any attention to the eggs. When all four specimens were killed a few days later for the purpose of determining the sex by dissection, the stomachs of the males were found distended with undigested eggs.

Upon examination, the female, which during captivity had laid nearly 400 eggs, was found to contain, at a rough estimate, seven or eight hundred more, in a state of development which indicated that had she been allowed to live they would all have been laid during the same season. Evidently the eggs are, in some cases at least, matured and laid in batches of a few hundred at a time.

According to Gadow ('01), *Amblystoma* alone among the Urodeles lays as many as 1,000 eggs in a single season. The facts stated above make it probable that *Cryptobranchus* rivals *Amblystoma* in the number of eggs laid. Kerbert's specimen of *C. japonicus* is reported to have laid 500 eggs in the fall of 1902, and about 900 in 1904. The eggs of *Amphiuma* found by Hay numbered about 150.

The absence of any evidence of brooding habits of either the male or the female *Cryptobranchus* is rather unexpected in view of the possession of brooding habits by closely related forms.

Concerning the nesting and brooding habits of *C. japonicus* Ishikawa says: "Das tier legt seine Eier in tiefe horizontal verlaufende Löcher, in denen das Wasser sehr ruhig ist. Manchmal ist solch ein Loch 10 oder mehr Fuss tief und kaum für das Licht zugänglich. Die Brutstellen für die Eier sind aber nicht immer so tief. Oft fand ich Eier in einem Loch nicht tiefer als 3 oder 4 Fuss. Oeffnet man ein solches Loch, so findet man eine abgerundete Stelle, deren Boden ganz rein gehalten ist. . . . Fast in jedem Loch, wo man von Ende August bis zu Anfang October ein weibliches Tier gefunden hat, findet man einen Eiklumpen. Dieser Umstand lässt schon vermuthen, dass das Tier eine Brutpflege hat wie *Ichthyophis* oder wie so viele andere Amphibien." Kerbert, however, asserts that it is the male that guards the eggs, and states that the sex of his specimens was carefully determined. The only external distinction between the sexes is that during the breeding season the lips of the cloaca of the male are greatly swollen. Ishikawa gives an illustration of an adult specimen of *C. japonicus* lying in a coil about a mass of eggs; Kerbert states of his specimen that the male, after driving away the female and also the small fishes present in the aquarium, crept between the folds of the mass of eggs, or sometimes simply lay down beside them, but in either case he kept the entire mass in motion by a pendulum-like movement of the entire body.

The eggs of *Amphiuma* found by Hay ('88 and '90) in an Arkansas swamp were in a comparatively dry situation, in a small excavation under a log several rods from the nearest water. The female was found coiled about the mass of eggs, protecting them and keeping them moist.

Other Amphibia for which brooding habits have been established are the Urodele, *Desmognathus*; the Gymnophiona, *Ichthyophis* and *Hypogeophis*; *Alytes* and several other Anura (Wiedersheim, '00). In the cases of *Desmognathus*, *Ichthyophis* and *Hypogeophis*, the female is said to care for the eggs; in the case of *Alytes*, the male.

E. *Adaptation of the Egg to its Environment.*— The egg proper is so soft and fragile that it can sustain only the most careful manipulation without injury; on account of its lack of firmness it soon becomes oblate from the effects of gravity. In view of

the fact that the eggs are laid in running water, the protection of such an egg from shocks and jars due to impacts against the rocks is an exceedingly important matter, and we find it accomplished by an admirable adaptation of the egg envelope. The tough but elastic egg capsule is inflated by osmosis with a liquid in which the egg freely floats. The turgid condition of the envelope increases its efficiency in protecting the egg. While ordinarily the egg sinks to the bottom of the surrounding liquid, and rests lightly upon the membrane beneath, there is so little friction between the egg and its envelopes that the latter may be rotated without turning the egg. A similar adaptation exists in the eggs of other amphibians, and in the eggs of teleosts (Reighard, '93); but it is particularly well shown in the egg of *Cryptobranchus*. The connecting cord is strong enough to support the weight of half a dozen eggs suspended out of water; it stretches greatly during the operation, but contracts to its usual length when the eggs are returned to the water. This elasticity of the connecting cord serves to deaden any shock to the eggs due to the tugging of the current or to the chain catching on to rocks while floating down stream.

The egg envelopes further serve to protect the eggs from little fishes that would otherwise devour them.

If eggs are left for several days undisturbed in the still water of a basin, some of them adhere to the envelopes beneath, interfering with the development. Probably under natural conditions the gentle agitation of the current tends to prevent this, performing for these eggs the same service afforded to the eggs of the hen by the mother when she turns them.

III. Segmentation.

The perfect transparency of the gelatinous envelopes of these eggs makes them very favorable material for watching the development of the living egg, since the process may be observed without removing the egg from the capsule. The large size of the eggs and the distinctness of the cleavage lines on their upper hemispheres also make them convenient objects for study. By using a mirror both poles of the egg may be watched at the same time, but observations on the lower hemisphere by this method

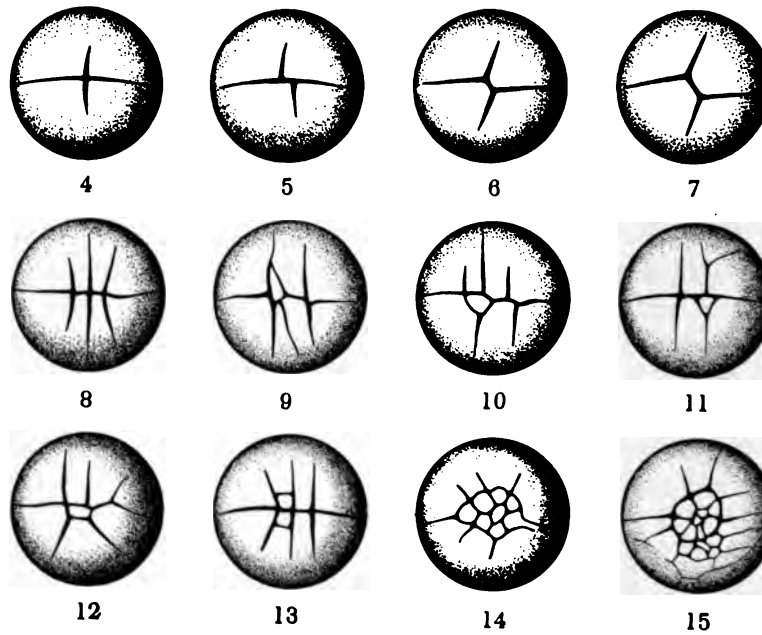
are rather difficult, on account of the faintness of the cleavage lines in this region. Material was preserved at intervals and camera drawings afterwards made of the principal stages; but for the earliest stages the freehand sketches made from living material are sufficiently accurate, and have been used in preference to camera drawings from preserved material.

Some of the eggs found on September 7 were left to develop *in situ* as a check upon those studied under artificial conditions; but so far as cleavage is concerned no difference could be detected between these eggs and those kept in a dish of shallow water.

The abundance of the material at hand made it possible to secure a series of drawings of the segmentation stages which should be fairly representative. The drawings include some of the most regular and geometrically perfect figures that could be found, and probably these may be regarded as typical; but in *Cryptobranchus* as in other amphibians the amount of variation is great. Since departures from the type are not necessarily the result of pathological conditions, they should not be dismissed as abnormalities; for they may be the expression of opposing factors in the development of the egg. Such factors are the proportion of yolk to protoplasm, the qualitative composition of the yolk determining the extent to which it responds to the sorting influence of gravity, and hereditary factors in the protoplasm; variations in these factors affect the manner of cleavage, and according as one tendency or another prevails we may find the typical form or variations from it in definite directions. Hence the figures include representations of eggs in the same stage showing differences in the method of cleavage. There are of course individual differences in the rate as well as in the manner of cleavage.

A. *The First Cleavage Furrow.* — In eggs laid September 6 at about 6:30 P. M., there was so much diversity in the time of appearance of the first cleavage furrow that it is difficult to assign limits. Probably more cases of first cleavage were noticed at about 10 A. M. of the next day — 15 hours after the eggs were laid — than at any other time. In eggs laid during the night of September 7–8 the first cleavage furrows appeared in several eggs at about 5 P. M. of the next day, and cases were numerous at 6:30 P. M. The eggs found on September 7 at 10 A. M. were

in the first cleavage stage; observations on the rate of deposition of silt on their envelopes indicated that they had been laid during the preceding night or late in the afternoon of the day before.



FIGS. 4–15. Early segmentation stages of eggs of *Cryptobranchus*. Freehand sketches from living material magnified about ¼ diameters.

FIG. 4. Egg found September 7 at 10 A. M., sketched at 1 P. M. The first cleavage line extends over exactly half a circumference.

FIGS. 5–7 show variations in the second cleavage. Fig. 5, egg laid September 6 at about 6:30 P. M., sketched September 7 at 3 P. M. Figs. 6 and 7, eggs found September 7 at 10 A. M., sketched at 1:30 P. M.

FIG. 8. Egg laid during night of September 7–8, sketched September 9 at 5:30 A. M.

FIGS. 9–11. Variations in third cleavage. Eggs laid during night of September 7–8, sketched September 9; Fig. 9 at 3:50 A. M., Fig. 10 at 2:30 A. M., Fig. 11 at 5:30 A. M.

FIG. 12. Egg laid during night of September 7–8, sketched September 9 at 5:45 A. M.

FIGS. 13 and 14. Fourth cleavage stages. Fig. 13, a later stage of egg represented in Fig. 8, sketched September 9 at 8 A. M.; Fig. 14, a later stage of egg represented in Fig. 9, sketched September 9 at 8 A. M.

FIG. 15. Egg found September 7 at 10 A. M., sketched September 8 at 8:45 A. M. Four nearly equidistant cleavage lines reach almost to the center of the lower pole.

It is not known whether the eggs of a given spawning are all fertilized at the same time, hence some of the diversity in the time of appearance of the first cleavage furrow may be due to a difference in the time of fertilization.

The first cleavage furrow begins as a pit, which gradually elongates, rapidly at first then more slowly. The point of origin of the first cleavage furrow remains always uppermost, even though the envelope is rotated.

B. *The Second Cleavage Furrow.* — This furrow makes its appearance about six hours after the first, which by this time has extended over about half the distance to the equator of the egg. The second cleavage furrow usually cuts the first at right angles, but some variations from this procedure are shown in the figures. (See Figs. 4–7.)

Some time before the appearance of the second groove, the first one appears constricted and narrow in the middle portion, while still broad at the ends. The earliest indication of the second furrow is usually a roughness in the region where the second groove is to intersect the first. The appearance of "Faltenkränzen" — a quivering of the surface with the formation of fine radiating or parallel wrinkles, which extend outward from the cleavage furrow for a moment, tremble, and disappear — a common phenomenon in the cleavage of the amphibian egg, is quite marked at the time of the beginning of the second cleavage furrow. For some time after its appearance the second furrow is much broader, though of course shallower, than the first.

C. *The Third Cleavage Furrow* (see Figs. 8–12). — These furrows mark the establishment of the 8-cell stage about four or five hours after the appearance of the second cleavage groove. Hence the third division is more rapid than the second. At the time when the third furrows are initiated the first furrow has usually reached or passed the equator; the second one is confined to the upper hemisphere.

The third cleavage furrows ordinarily begin as two pits in the first furrow, not far from its point of intersection with the second — the center of the animal pole of the egg — and equidistant from that point. From these pits the third furrows proceed in an approximately vertical direction. The third cleavage furrow seldom

originates from the center of the first furrow, and seldom reaches the lower pole, but extending obliquely in the lower hemisphere joins the second furrow at some distance from the pole (see plate, Fig. 16*B*, line *b*; Fig. 17*B*, lines *b*, *g* and *e*; Fig. 18*B*, lines *b*, *i* and *e*, etc.). Hence the third cleavage furrow is *intermediate between a true meridional cleavage and a latitudinal one*, approaching more nearly the meridional type. This will be made clearer by supposing the point of origin of the third cleavage furrow to be shifted along the first further from the animal pole; and its point of junction with the second to be shifted further from the lower pole; the third groove will then become latitudinal. On the other hand, if the two ends were shifted in the reverse directions, the cleavage lines would become truly meridional. Variations from the customary mode of cleavage tend to confirm the view that it is an intermediate or transitional form; while some third cleavage furrows originate at the center of the animal pole, giving a true meridional cleavage, others come in latitudinally. (See Figs. 9–12.)

D. *The Fourth Cleavage Furrow* (Figs. 13–15). — This comes in about 3 hours later — a briefer interval than that preceding the appearance of the third cleavage furrow. In its typical condition it is parallel to the equator, but close to the animal pole, so that the division is very unequal. Irregularities in this cleavage are very numerous.

E. *Later Segmentation Stages* (see plate, Figs. 16–24). — The later divisions occur with increasing rapidity, but with such irregularity that no definite arrangement of cleavage furrows can be made out. Cleavage proceeds rapidly near the animal pole, much more slowly in the equatorial region, and is greatly retarded in the lower hemisphere. At some time during the second day after the egg is laid the first cleavage furrow reaches the lower pole. At this time the upper hemisphere is cut up into a considerable number of cells. The macromeres continue to be very much larger than the micromeres. While so far as can be determined from a surface study of the egg, the cleavage is undoubtedly holoblastic, a strong tendency toward the meroblastic condition is evident.

Two days after the egg is laid, cell division in the upper hemisphere has advanced until the individual cells can no longer be seen with the naked eye. The macromeres are in general still quite large, but in several instances there was noted a greater multiplication of cells about the lower pole than in the equatorial region (see Fig. 24*B*).

F. *Comparison with Other Forms.* — De Bussy ('05) has described the principal segmentation stages of the eggs of *C. japonicus*. His material lacked eggs in the 2-cell and 4-cell condition; but beginning with the 8-cell stage the later cleavage stages are described and illustrated. A comparison with my own sketches of the segmentation of the eggs of *Cryptobranchus alleghenicnsis*, made before I knew of the existence of de Bussy's paper on *C. japonicus*, shows, as might be expected from the close relationship of the two forms, a marked similarity in the mode of cleavage. Since de Bussy's paper includes a comparison of the eggs of *C. japonicus* with those of other forms, a similar discussion for *C. allegheniensis* at the present time seems unnecessary. It should be added, however, that the segmentation of the eggs of *Desmognathus*, described by Wilder ('04) and Hilton ('04) also bears a considerable resemblance to that of *Cryptobranchus*.

I take pleasure in acknowledging my indebtedness for encouragement and advice to Prof. Jacob Reighard, at whose suggestion I first looked for the eggs of *Cryptobranchus*, and to Dr. O. C. Glaser, under whose direction the work was carried on after I returned to the university.

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EXPLANATION OF PLATE.

All the figures are camera drawings from preserved material. Figs. 16–19 are magnified about three diameters, the remaining figures about four diameters. *A* and *B* indicate upper and lower hemispheres respectively. The small letters serve to identify lines which cross the equator.

FIGS. 16–18. Eggs laid during night of September 7–8, preserved September 9 at 2 P. M.

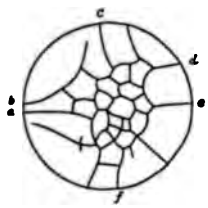
FIGS. 19 AND 20. Eggs found September 7 at 10 A. M., preserved September 8 at 9 A. M.

FIG. 21. Egg laid September 6 at 6:30 P. M., preserved September 8 at 8:30 A. M. Lateral view.

FIG. 22. Egg laid during night of September 7–8, preserved September 11 at 10 A. M.

FIG. 23. Egg found September 7 at 10 A. M., preserved September 10 at 8 A. M.

FIG. 24. Egg laid September 6 at 6:30 P. M., preserved September 8 at 8:30 A. M.



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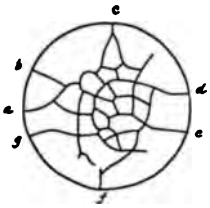

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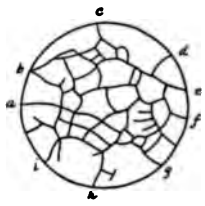

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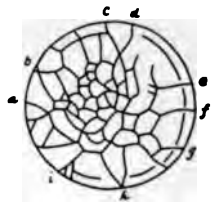

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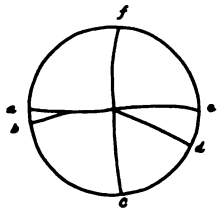

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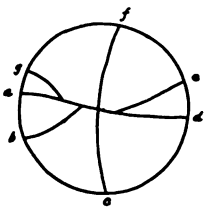

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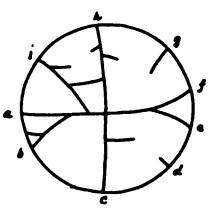

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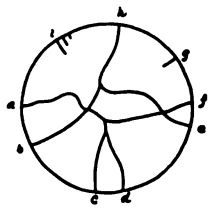

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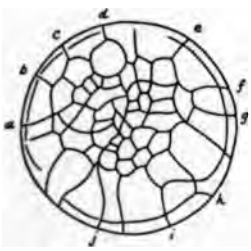

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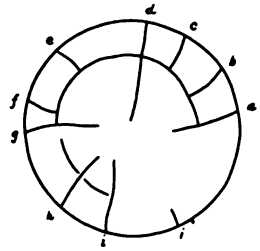

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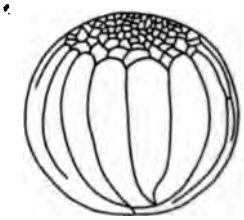

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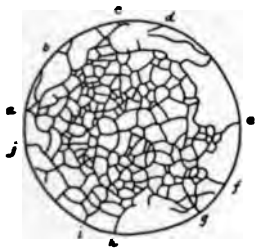

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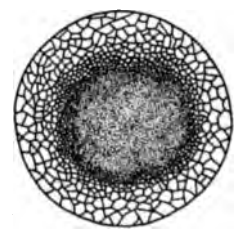

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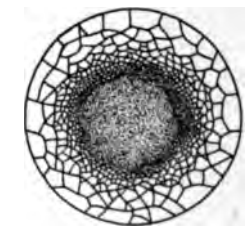

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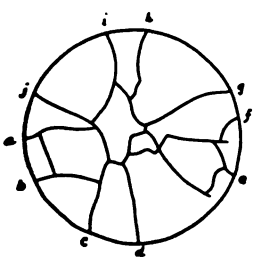

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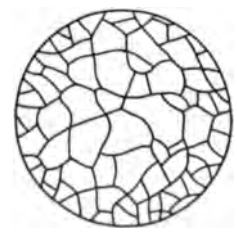

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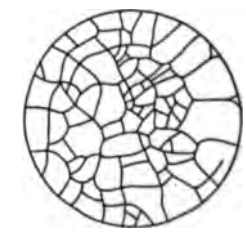

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BIOLOGICAL BULLETIN

OF THE

Marine Biological Laboratory

WOODS HOLL, MASS.

VOL. XI SEPTEMBER, 1906 No. 4

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BIOLOGICAL BULLETIN

SOME CONSIDERATIONS REGARDING SO-CALLED FORMATIVE SUBSTANCES.

C. M. CHILD.

Within the last few years the idea of "formative substances" has received consideration and support from workers in certain fields of zoölogy and embryology to such an extent that it appears worth while to examine critically some of the assumptions upon which the hypothesis of the existence of such substances rests.

As the first step in this consideration a definition of formative substances is necessary. But anything like an exact definition is difficult to find even in the literature which has devoted most attention to these hypothetical substances. We read of ectodermal substance, myogenic substance, hydranth-forming substance, head- and tail-forming substances, etc., but the general question, what is a formative substance? does not seem to be very clearly answered. It may, however, be inferred from the use of the term that a formative substance is a specific substance capable of giving rise under certain conditions to a specific structure. Secondly, we must inquire as to the nature of organic structure to which these formative substances are supposed to give rise? Organic structure may be defined as a complex of heterogeneous parts of typical constitution and arranged in typical space relations. Strictly speaking this definition is not exact, for organic structure, like other kinds of structure, is fundamentally simply a matter of space-relations or arrangement of parts entirely independent of their constitution. The constitution of the component parts must of course play a rôle in determining the dynamic or functional activities of the structural complex, and ordinarily we do not

clearly dissociate the idea of organic structure from the dynamic activities which it exhibits. For the sake of critical analysis, however, it is necessary to emphasize the fact that organic structure is primarily a typical arrangement of heterogeneous components without regard to their constitution. This becomes evident at once from the following consideration: if each component of a given organic structural complex could be distinguished it would be possible to build up artificially an identical structure in which each component was different in constitution from the corresponding component in the original structure. On the other hand, if all the different components of an organic structural complex could be isolated without altering their condition and then mixed together the mixture would not represent the original structure.

It thus becomes evident that the space-relations of the components, not their constitution, are the characteristic feature of structure proper. From this conclusion it follows that formative substances, if they exist, must be specific substances which determine the arrangement of the component parts of a structural complex. In order to determine whether formative substances do exist we have first to consider the possible methods of origin and action of such substances. As regards the individual, for example, each substance must either be represented in the primitive germ-cell by an ultimate "unit of organization" of the same nature as itself, or it must have arisen from other substances in the course of development.

In the one case development is merely the "unfolding," "activation," or whatever we prefer to call it, of preformed "germs," "determinants," or "definite, determinate and primary" units of organization. In the other case it is, properly speaking, an epigenesis, in the course of which new formative substances appear as the result of the activity of others, and the complexity of the developing organs increases.

Directing our attention first to the preformation hypothesis we must inquire as to the nature of these ultimate units which represent both the formative substances and the structures of later stages. Are they simply molecules or groups of similar molecules, or are they something more complex?

Ultimate Units Molecules or Groups of Similar Molecules.

If the ultimate units are molecules or groups of like molecules, then their properties as formative substances must be the result of their physical and chemical properties as molecules. If this be admitted the hypothesis based on these assumptions is identical with or similar to the crystallization hypothesis. For structure is primarily a definite and characteristic space-relation of parts and an aggregation of like molecules cannot, so far as we know, give rise to anything like definite and characteristic fixed space-relations except by an arrangement of its molecules according to physical laws. According to this view organic structure is made up of a multitude of component parts, each consisting of like molecules, but each different from the others in composition and each arising from its corresponding ultimate unit. But in the fully developed structure these different components are definitely arranged, and indeed it is this feature — the definite space-relations of heterogeneous parts — that is the fundamental characteristic of all structure. To what is this arrangement due? If we say it is due to the physico-chemical interactions and relations to each other of the substances in a given environment, then we have departed from our premises, for in this case the relations and the environment are more directly and immediately formative factors than the substances. The substances have formed the various components, but there is no substance which can be made to account for their arrangement.

If, on the other hand, we assume that the characteristic arrangement of the heterogeneous parts is due to something else than their physico-chemical interactions and relations in a given environment, then we pass beyond the realm of physics and chemistry and become "vitalists."

In short if we start from a series of molecules or groups of like molecules as the ultimate formative substances, we can, by assuming a process of crystallization or something similar in each substance, account for the development of each physico-chemically homogeneous element of structure, but we cannot account for the most important feature of structure, *viz.*, the characteristic arrangement of heterogeneous elements except by assuming rela-

tions and interactions as the formative factors or by adopting a vitalistic point of view. In any case the formative substances are inadequate to produce the observed result.

But the crystallization hypothesis has found no wide acceptance. Most authorities agree that the process of formation of the various parts of organic structure is at least in most cases either only very remotely similar to the process of crystallization or differs totally from it. It may be that in certain cases the process of morphogenesis is akin to crystallization but the results of experimental work demonstrate that in very many cases it certainly is not.

Ultimate Units Groups of Dissimilar Molecules.

The other alternative under the preformation hypothesis, *viz.*, that which assumes the ultimate units or formative substances to be something more than molecules or groups of like molecules, also presents certain difficulties.

These complexes must be either simply physical mixtures or complexes or they must be molecular complexes of some other kind.

If we consider them as physical complexes then their formative activity is the result of the relations between parts or of these relations plus a given environment, *i. e.*, none of the substances can be regarded as in itself formative but only the complex as a whole or the complex plus the environment.

Now if the formative substance is a mixture or other physical complex whose activity depends only or primarily on relations between its parts why does its activity appear only at a certain time? If we admit that the environment, *i. e.*, relations with other elements, etc., play a determining rôle then these relations are just as truly formative as the complex on which they act. Moreover, as in the case considered under the previous heading we must either assume that the characteristic arrangement of the elements in developed structure is due to physico-chemical relations between the different complexes or we must take refuge in vitalism. But let us assume that the ultimate element is a complex of molecules of some other sort, *i. e.*, that it possesses a certain "organization." Physics and chemistry recognize at

present no such organization of dissimilar molecules. Thus we are again in danger of being regarded as vitalists. Even if we assert that this organization is finally reducible to a physico-chemical basis we cannot show how it is reducible. In any case we are adopting a position for which the facts afford no warrant; moreover, we are assuming in our hypothesis exactly that which the hypothesis is supposed to explain, *i. e.*, we postulate one organization in order to explain another. Naturally if organization forms the basis of the hypothesis no real difficulties will be encountered in individual cases for we can refer them at once to the hypothetical organization. One frequently reads that a considerable degree of organization is necessary for the phenomena of life, that protoplasm possesses a high degree of morphological organization, etc. The authors of these statements do not always inform us as to the nature of this organization, but there is, I think, no escape from the conclusion that unless we admit that a molecule or a physical complex of molecules can exhibit the phenomena of organic life our hypotheses are open to the objections stated above.

In his earlier work Weismann designated the ultimate units postulated in his hypothesis as molecules but later asserted that they are not molecules in the physical sense, but consist of a number of such molecules. Unless these complexes are purely physical, and so far as I can determine, Weismann does not seem to regard them as such, his hypotheses is open to these same objections. Other similar "organization" hypotheses are in the same position.

The Hypothesis of Epigenetic Origin of Formative Substances.

If a development of new formative substances from previously existing substances occurs during development we come ultimately as regards ontogeny to the primitive germ-cell which must be composed either of a formative substance or of a complex of substances from which the substances appearing in later stages arise. At the present time probably no one would regard the primitive germ-cell as containing only a single formative substance but such a hypothesis would be a contradiction in terms

since the real formative activity would in this case reside in the environmental conditions not in the substance.

Assuming then that the primitive germ-cell contains a complex of formative substances all that has been said in the discussion of preformation hypothesis regarding the nature of ultimate units of organization would apply here. The complex must be physico-chemical in nature and the formative factors must reside in the relations between the constituents of the complex and in the environment, not in the single constituents themselves, for a single substance cannot of itself give rise to unlike substances, definitely arranged.

Most hypotheses of this character assume further that the elements of this ultimate complex are definitely and characteristically arranged. This arrangement must be the result of physico-chemical conditions past or present, *i. e.*, the cause of the organization is to be found in relations not in particular substances, or else we must again take refuge in vitalism and assume the existence of some organizing principle ultra-physico-chemical in nature.

The next question to be considered is — how do the new formative substances which appear at any given stage and in typical space relations arise from those previously existing? Here again we must point to the physico-chemical conditions in the complex, *i. e.*, to the relations and interactions of the elements in a given environment as the real formative factors, since a single substance cannot of itself give rise to unlike substances in typical space-relations.

And finally, how do the last series of formative substances give rise to the definitive structures? Here three points of view are possible. We may assume as in the preformation hypothesis a process of crystallization or something similar but by so doing we account only for the form of the elements of structure not for the characteristic arrangement of these elements. We may attempt to account for this by assuming that the formative substances existed in a similar characteristic arrangement, but this only throws the difficulty back to earlier stages. We must turn sooner or later from substances to relations or else to vitalistic assumptions. Secondly we may assume some grouping principle

of unknown nature — again a vitalistic conception — or finally we must admit that the relations not the substances give rise to the final arrangement of parts.

THE RÔLE OF CHEMICAL CONSTITUTION IN MORPHOGENESIS.

Objection may be made to the conclusions reached above on the ground that the chemical constitution of the various substances plays a part in determining the character of the relations which exist between them, and consequently in determining the character of the result. It is of course quite true that the chemical constitution of the substances involved may affect the result, but a single substance can accomplish nothing of itself by virtue of its composition. It must enter into relation with another or others in a more or less typical environment and the composition of the other substances and the factors of the environment are equally important in determining the result. What are the real formative factors in this case? It is evident that we can properly recognize only a formative complex of substances and relations and in this complex the constitution of the various substances is a condition affecting the character of the result while the relations between the substances are the cause of the result. But again it may be objected that the chemical constitution is the cause of the relations existing. While this may be true in large measure for certain simple chemical reactions it certainly is not true for many of the processes which give rise to organic structure.

Many other conditions beside those resulting from chemical constitution are usually important. For example, we know that the formation and persistence of various structures is determined primarily by simple mechanical conditions. It may be said that these conditions are not effective unless they act upon a particular substance but unless we make the term particular substance equivalent to protoplasm of a particular species or genus there is no evidence that this is true.

There is very strong evidence for believing that mechanical conditions resulting from the presence of fluid in a cavity are often fully as important as the character of the cells about it in determining the development of hollow, fluid-containing structures. We know that mechanical conditions are potent factors in the de-

velopment of bone and connective tissue and many other similar cases might be mentioned. It is conceivable that in some formative processes the most important conditions are chemical relations, but even in such cases the chemical constitution of a given substance is only indirectly "formative."

Moreover, in many other dynamic phenomena occurring both within and outside of the organism the chemical composition is of little importance. The catalytic activity of colloid solutions of metals does not appear to differ fundamentally from that of enzymes and yet the substances involved are widely different; in many of the effects produced by electrolytes one chemical element or radical may be substituted for another without altering the result: compounds of widely different chemical composition may produce identical osmotic phenomena, etc. In short, chemical constitution is at best only one of a large number of factors involved and often is of little or no importance.

Morphogenetic properties have been assigned by various authors to specific enzymes and to other substances of more or less definite chemical constitution. For example, in a recent paper[1] L. Loeb makes the following statement: "Ferments produce primarily chemical changes. But we know of chemical ferment actions which bring about structural changes in the medium in which they act. Thrombin in transforming fibrinogen into fibrin changes a colloidal fluid into a gelatinous, more or less solid mass, which under the influence of pressure and traction may show a fibrillar structure not unlike connective tissue. From a certain point of view the fibrin ferment may therefore be regarded as a form-producing ferment. We might call it a morphogenetic ferment. We have reason to assume that there exist other morphogenetic ferments" (p. 150).

A critical examination of this statement will at once render it evident that the real formative factors in the production of the fibrillar structure are the pressure and traction, and not the enzyme. The enzyme simply changes the condition of the medium in which it acts in such manner that pressure and traction produce a visible structural effect. But the enzyme itself is not properly speaking morphogenetic. The other cases

[1] Immunity and Adaptation. BIOL. BULL., Vol. IX., No. 2, 1905.

of so-called morphogenetic enzymes seem to be similar to this one in that the structure appears, not as the result of enzyme action, but in consequence of certain physical conditions which may accompany or follow the enzyme action but are not an essential part of it. There is nothing specific in the relation of this process to the enzyme. Coagulating or coagulated colloids may be made to assume a fibrillar structure in many cases "under the influence of pressure and traction" in total absence of enzymes. The specific effect of the thrombin and of other enzymes as well has not in any case been shown to be morphogenetic in character.

In general, physical conditions appear to be more important factors in morphogenesis than substances of particular chemical constitution.

Formative Substances in Ontogeny.

The chief reason for the consideration recently accorded to this old idea of formative substances appears to lie in the fact that many eggs exhibit visibly differentiated regions which normally give rise to particular structural complexes. Moreover, experiment has demonstrated that in some cases certain of these regions are capable of continuing the process of visible differentiation in a manner apparently normal after separation from other parts of the egg or embryo. The conclusion in such cases is that these regions must contain a certain substance or certain substances which are responsible for the differentiation. Hence these hypothetical substances are called formative. These regions are mostly extensive and give rise in development to a multitude of structures : for example we read of ectodermal formative substances, neurogenic substances, myogenic substances, entodermal substances, etc. In some cases, however, smaller regions giving rise to definite organs or parts of organs appear to possess in greater or less degree the power of "self-differentiation." We must regard these regions from either the preformation or the epigenetic point of view, *i. e.*, each element of the structure to which they finally give rise must be represented by an element existing before visible differentiation or else new elements must arise in the course of development. In any case the region must

be a complex. All that has been said above regarding complexes of substances applies here. The result produced is the result of the sum total of conditions existing in the complex. When we assume that the result is due to the presence of certain specific substances in the complex which manifest themselves in a peculiar "formative" fashion we are not only making an inference not warranted by the facts, but we are involving ourselves in various difficulties which become manifest only on careful analysis. Some of these I have endeavored to point out above. All the substances in the complex are or may be formative, and under certain conditions the region gives rise to a characteristic differentiation. But if all the substances are formative the distinction of formative substances is entirely unnecessary and we must regard the region merely as a formative complex.

But it may perhaps be maintained that the formative substance hypotheses do not differ essentially from this since the formative substances are really complexes, not definite chemical substances. If this is the case then it is certainly preferable to avoid the use of the vague term substance in this connection, especially since "formative" factors are known in many cases to be related to substances only very indirectly. Moreover, it is difficult to understand the grounds for distinguishing a particular complex either of substances or of conditions as formative since this implies that others are not formative. The only conclusion justified with regard to "self-differentiation" in isolated blastomeres or egg-regions is that the conditions for the observed differentiation reside in the part. This, however, is really quite different from the conclusion that the piece contains certain formative substances for the particular structures to which it gives rise. The region may differ chemically in greater or less degree from other regions of the egg and this difference may be more or less closely related to the structural result and to that extent formative. But to dignify the chemical peculiarity of a region by the term formative substance involves unwarranted assumptions. The strongest evidence in support of formative substance hypotheses is found in these cases of ontogenic self-differentiation of parts and this, as has been seen, is far from conclusive.

In order to avoid misconception it should perhaps be stated

positively that the writer's position does not at all involve a denial of the clearly demonstrated fact that these regions are different or have become different from others in the egg or embryo, nor does he underestimate the value of the observations which have directed attention to this fact. But to say that these regions contain specific formative substances is to say too much, for the visible differences do not necessarily stand in direct relation to their formative activity. The same "kind" of protoplasm is certainly capable of widely diverse formative reactions under different conditions and the determining conditions are demonstrated in many cases to be dynamic rather than substantial. It seems more nearly correct therefore to maintain that particular lines of activity have been initiated in these formative regions in consequence of past or present conditions and may continue even after isolation of the regions. That different substances exist in the egg and embryo is also clearly demonstrated, but to assert that these are formative or that their presence indicates the existence of particular formative substances is quite another matter, and as the writer believes, not justified by the facts. Should we not therefore be content in view of the facts to designate these so-called "formative" or "morphoplasmic substances" merely as cytoplasmic differentiations, leaving the question as to the nature and significance of the differentiation open?

Formative Substances in Regulation.

The attempt has recently been made by Morgan[1] to account for certain phenomena of regulation, especially those connected with polarity by postulating the existence of formative substances. This hypothesis is selected for discussion since it is the latest attempt to interpret formative processes in this manner. Other similar hypotheses are, however, open to most of the same objections. In addition to the objections discussed above to which the idea of formative substances in general is open there are certain other objections which apply specially to the application of this idea to the phenomena of form-regulation.

It is of interest to note first that these formative substances are quite different from those of the embryologists. Here for exam-

[1] *Science*, XX., December, 1904. *Journ. Exp. Zoöl.*, I., 1904, and II., 1905.

ple instead of ectodermal, neurogenic, myogenic, mesenchymal, entodermal, substances, etc., we have head-forming and tail-forming substances, pharyngeal substances, hydranth-forming material, stolon-forming material, etc. How do these substances arise from the substances supposed to exist in ontogeny. A head or tail of a planarian or the hydranth of a hydroid is a very complex structure which has developed from regions more or less widely separated in the egg. Is the head-forming substance made up of all the different substances which were concerned in the original development of the head or is it something new? If it is the sum total of the ontogenetic substances then it must in itself be organized, *i. e.*, the different parts of which it consists must possess characteristic positions with reference to each other and we have another organization to account for. Moreover, why should such an enormous amount of head- and tail-forming substances as must exist for example in planarians be left over after development is completed? What is their function in the normal animal or are they without function? If the latter is the case it looks very much as if provision were made in the normal animal for regulation, in other words as if regulation might be after all an adaptation.

Polarity is regarded as identical with a postulated gradation of the various materials. The head-forming materials in planarians, for example, are supposed to decrease from the anterior end backward, the tail-forming materials in the opposite direction, the pharynx-forming material from the middle toward both ends, etc. This idea followed to its logical conclusion certainly does not assist us in comprehending organic structure, but rather increases the difficulty. How did this gradation come about and why should it exist? As we have seen, the various head-forming substances were widely separated in the egg. How and why have they combined in this peculiar manner? Why does not a small piece from the region of the body just behind the head in *Planaria* always give rise to heads at both ends, since the head-forming substance is greatly in excess at both ends as compared with the tail-forming substance? Why does not the pharynx in such a piece arise at the extreme posterior end since the pharynx-forming substance is more abundant there than elsewhere in the piece?

In considering the case of *Tubularia* Morgan[1] makes the following statement which may perhaps serve to illustrate the nature of his hypothesis: "We may assume that the gradation of the material is of such a kind that the hydranth-forming material decreases from the apical toward the aboral end. The formative influence acting from the exposed end inward (the stimulus of the water on the free end), finds a prompter response when it acts in the direction of decreasing amounts of hydranth-forming material (which has the same gradation as that in the hydranth itself) than when acting in the reverse direction (namely at the aboral end). Therefore the oral polyp, as a rule, develops first. For its development it needs certain nutritive material. This it finds either in the cœnosarc or in the circulation, and uses the materials as it develops. In consequence the cut surface at the basal end cannot get the material necessary for it to develop into a hydranth and it either remains undeveloped or produces a stolon."

Besides the assumption of the gradation of material this statement of the hypothesis contains a number of other assumptions. The rapidity of response to the stimulus depends on the direction of gradation of material; a gradation of hydranth-forming substance exists in the hydranth itself. The hydranth takes up nutritive material and so prevents the aboral end from obtaining it, so that this produces a stolon or nothing. But why should the rapidity of response depend on the direction of gradation? What reason is there for supposing a gradation of hydranth-forming substance in the hydranth itself? Why should a stolon develop because the aboral end cannot obtain the nutriment which is taken by the developing hydranth? Does the developing stolon require no nutritive material or does it require a different kind from the hydranth? If the latter, then stolon-formation and logically also hydranth-formation are due not to stolon-forming and hydranth-forming substances laid down in the stems in regular gradation but to nutritive substances occurring apparently anywhere. Moreover, we are told that the formative influence is the stimulus of the water on the free end. If this is the "formative influence" what need is there for hydranth-forming substance?

[1] *Journ. Exp. Zoöl.*, I., 4, 1904, pp. 587–588.

I find it very difficult to obtain any definite conception of the character of the hydranth-forming substance or of the manner in which it acts. Apparently it is not responsible for the size of the structure but only for its characteristic form. In the further development and discussion of the hypothesis a large number of special assumptions are made to meet special cases.

Loeb has recently asserted his belief in the existence of formative substances in connection with regulation and especially as determining polarity. Loeb's view appears to resemble the older Bonnet-Sachs hypothesis of the migrations of particular substances in particular directions. According to Loeb the formative substances are nutritive in character. This hypothesis does not appear to meet the objections to which other hypotheses are open and introduces some new difficulties since conditions determining the localization and distribution of the nutritive material do not seem to be considered.

On the other hand, various results have been obtained in work along this line which hypotheses of formative substances do not seem to me to account for. To mention only a few of these, in *Leptoplana* the larger the portion cut off posteriorly the larger the amount of new tissue formed at the posterior end of the piece remaining. Thus a piece comprising only the anterior third or fourth of the body will produce without being fed five or six times as much new tissue as the anterior four fifths. Yet the "tail-forming substances" and nutritive substances must be much more abundant at the posterior end of the longer piece than at that of the shorter. Again, of two pieces of *Leptoplana* with posterior ends at the same level one containing the cephalic ganglia produces a larger tail than one without them. Why should this difference occur, since the tail-forming substances are the same in amount at the level of the cut and the amount of nutritive substances must be approximately the same in both cases. In *Cestoplana* the new pharynx appears at a given level in a piece containing the ganglia and farther anteriorly in a piece from the same region without the ganglia.

Objection may be made to the citation of these cases here on the ground that they are not pertinent to the matter in hand, in that they are not connected with the problem of polarity. It seems

to me, however, that the relative size of structures produced from different levels and the relative position of intermediate structures are as truly an expression of polarity as the existence of different structures at the two ends, since they must be the result of characteristically different physiological conditions present at the different levels.

Though I have not attempted it, it is possible that with the aid of special assumptions these cases could be made to fit into a formative substance hypothesis, but the value of a hypothesis is to a certain extent inversely proportional to the number of special assumptions required, and there is always danger that a special assumption may introduce more difficulties than it removes.

Moreover, if axial polarity is due to a gradation of substances, we must logically conclude that a gradation of substances from the median line to the lateral margins of the body in both directions exists, for the structural differentiation in these directions is just as truly an expression of "polarity" as is the axial differentiation. This leads us into numerous further difficulties. This lateral gradation must exist in the developing parts as well as in the old, otherwise the differentiation between lateral and median regions could not arise. How, for example, are we to account for the formation of the other side of the body and of a median region in longitudinal pieces of planarians of less than half the width of the body? In whatever manner we may proceed to bring these cases under the general hypothesis certain other special assumptions are necessary. If we say that the formation of the opposite side along the cut surface is due to the excess of margin-forming substance in the piece, how shall we account for the formation of a median region between the two margins? If, on other hand, we attribute the formation of a median region to gradation of the formative substances for this region, supposing them to be in excess at the cut surface, how shall we account for the formation of the opposite side? Furthermore the new marginal region is formed first. Consideration of other experimental data adds further difficulties.

But perhaps enough has been said to show some of the special difficulties in which a hypothesis of formative substances involves

us when we attempt to apply it to the phenomena of form-regulation. Some of the special cases discussed by Morgan will be considered more fully elsewhere in connection with new experimental data.

Such a hypothesis appears to me a totally unnecessary assumption in most cases, probably in all. Moreover, it does not afford a satisfactory explanation of the facts. I have recently made an attempt to account for some of the facts of regulation in a somewhat different manner,[1] and without assuming the existence of a series of substances which seem to me to make comprehension more difficult.

The Nature of Formative Processes.

The chief peculiarity, and, as I believe, the fundamental error in the hypotheses of formative substances seems to be that they regard the process of morphogenesis as something *sui generis* and not as simply a part of the dynamic or functional activity of the organism. The activities of the organism are considered as two-fold, one group being concerned with the construction of a complex machine, the other with the functions of that machine when completed. According to this view development is primarily a period of construction and not of function. This idea is perhaps a natural consequence of the separation of morphology from physiology. But when we consider the data already at hand it seems impossible to make any such distinction. The organism is primarily and at all times a dynamic or functional complex and the process of morphogenesis is merely an incident, or, in other words, structure is a visible by-product of these activities. In short, it is rather the result of the relations of parts than of any "formative" capacity existing in the single elements themselves, and as regards any given element or unit of the organism the factors of the environment and not the element itself must be, as we have seen, in the final analysis, the real formative factors. These environmental factors may be otherwise designated as the functional or dynamic conditions and we may regard development as primarily a functional process.

[1] *Roux's Archiv.*, XX., 3, 1906.

It is, of course, quite true that the character of the dynamic or functional conditions at any given stage is determined in greater or less degree by the character of the structural consequences of previous activities. These new activities give rise to new structural results until a certain stage is attained, determined doubtless by the complexity of the physico-chemical constitution of the organism, where the mutual determination of structure and dynamic conditions maintains a more or less exact equilibrium for a time. At this stage development is said to be completed. Any or all of the dynamic conditions may in proper environment give rise to conditions visible as structures, hence there are no good grounds for distinguishing certain groups of them or of the substances or structural complexes in connection with which they arise as formative from other merely "functional" groups.

HULL ZOÖLOGICAL LABORATORY,
UNIVERSITY OF CHICAGO,
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THE FORMATION OF NEW COLONIES OF THE ROTIFER, MEGALOTROCHA ALBO-FLAVICANS, EHR.

FRANK M. SURFACE.

Colony formation is not common among the Rotifera. But in several species, belonging especially to the family Melicertidæ, the individuals do become aggregated into colonies. These animals do not reproduce by budding, as do so many colonial forms, nor in most cases does the colony contain the progeny of its members. Instead, the young are hatched as free swimming individuals which later become segregated into a separate fixed colony.

During the past winter, while a graduate student at the University of Pennsylvania, the writer made some observations on the formation of new colonies of *Megalotrocha alboflavicans*. Colonies of this large rotifer were found in abundance in the fresh-water tanks of the vivarium, whither they had been transferred some years before from the small pond in the botanical gardens. These colonies are ordinarily formed on the roots and stems of various water plants, but in the vivarium they were found only on the stems of *Myriophyllum*. The colonies are nearly spherical in shape and when adult may measure as much as 4 mm. in diameter. They are thus easily recognizable to the unaided eye, appearing as white spots on the dark or green stems. The colonies used were kept in glass jars in the laboratory and for observation a piece of the stem containing a colony was removed, placed in a small dish and observed with a Braus-Drüner binocular.

The work was undertaken at the suggestion of Professor E. G. Conklin, and it is a great pleasure to acknowledge my indebtedness to both him and Professor H. S. Jennings for their many valuable suggestions and kindly criticisms.

The colonies of *Megalotrocha* are not surrounded by gelatinous masses or tubes as are certain other species of the Melicertidæ.

The animals are however attached to the stem by a kind of mucilaginous substance secreted by a gland in the foot. This substance forms a thin layer on the stem over the area of attachment of the colony. The adult colonies usually contained eggs or at least soon produced them after being brought into the laboratory. The eggs are attached to the mother by means of an adhesive gland situated a short distance posterior to the opening of the cloaca. Usually only one or two eggs are found attached to a single individual, but sometimes as many as four were observed.

The method of egg deposition is interesting. The egg rapidly increases in size in the region of the vitellarium, and then passes slowly down the oviduct to the cloaca. When the egg with its large germinal vesicle has reached this region the animal bends towards the dorsal side in such a manner that the adhesive gland touches the protruding egg. The animal frequently remains in this position for some time, often bending still farther so that the corona points towards the foot. In this way the end of the egg is firmly pressed against the adhesive surface of the gland. The animal now slowly and by repeated attempts straightens itself and at the same time the egg is pulled from the cloaca and remains attached to the mother, where it undergoes development. One egg has scarcely been deposited before another can be seen enlarging in the region of the vitellarium. Eggs begin to form in all the individuals of a colony at approximately the same time. But an interval of three or four hours may elapse from the time the first egg is laid until all the animals have deposited eggs. During this time the individuals which deposited the first eggs have often deposited a second. This overlapping of broods is important, as will be pointed out later, in keeping the size of the colonies more nearly constant.

The length of time required for the young rotifers to hatch varies somewhat, depending on the temperature and other conditions. Usually they hatch in three or four days after deposition. The young rotifer when fully formed can be readily seen through the transparent egg membranes. For some hours before hatching frequent contractions of the body and movements of the cilia and mastax may be seen. By means of these contractions the

young animal finally bursts the enveloping membrane and is able to swim about. The young rotifers possess an organization in most respects similar to the adult. But among the more important differences may be mentioned the following. The trochal disc is at this time no broader than the trunk, the whole animal tapering slightly towards the foot. At the foot there is a circlet of small cilia. On the anterior border of the trochal disc, near the dorsal side are two red eye spots which are lacking in the adult. The cement gland in the foot is proportionately larger in the young animal than in the adult.

The young rotifers are free swimming, but are always attached at the posterior end by a thread of adhesive material which they spin out much after the fashion of a spider. They swim about among the adult rotifers, sometimes venturing a short distance beyond the limits of the colony but always drawing back and continuing to move about among the old animals. During all this time they are attached to the old colony by the adhesive thread from the foot. By a continuation of this nervous crawling and swimming some of them finally get their webs so twisted together that they are brought into contact. Here they remain, apparently without secreting more of the adhesive thread. At first there are only two or three individuals thus approximated and these are near the center of the old colony. Soon other young rotifers get their webs entangled with that of the few aggregated ones and these are then added to the ball that is forming. Here as well as later, contact with the adhesive thread appears to act as a guiding stimulus to the young rotifers. As soon as a young animal comes in contact with the rather thick thread leading to the forming ball, it at once begins to move up or down this thread until it finally reaches the ball; this I have repeatedly seen. Usually the young animal does not at once attach itself to the ball, but continues to crawl about, often through the bunch of young rotifers. In this way it finally becomes attached to its comrades with its foot closely adhering to theirs. During all the time that the ball is forming, the individuals composing it act in a very nervous manner, constantly jerking, twisting, contracting and expanding in a most irregular way. By means of this continual twisting and squirming the forming ball succeeds

in stretching the thread holding it fast, so that later it comes to lie outside the limits of the old colony (Fig. 1). In the course of time this thread breaks and the young ball swims away.

From the time the first individuals are hatched until the ball breaks loose there is usually a lapse of three or four hours. Since all the eggs of one brood are not deposited at the same time the period of hatching often extends over several hours. The long time that it takes for the ball to form and break away gives opportunity for the later individuals to hatch and get into the new colony. Often there are a few young that do not get

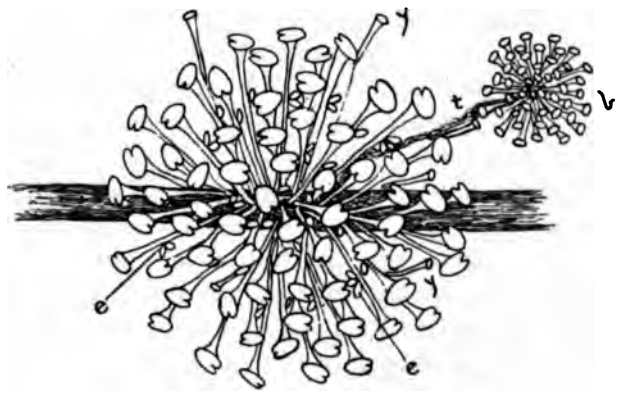

FIG. 1. Sketch to show formation of the swimming ball. *e*, eggs, attached to the adults; *y*, young animals before coming into the ball (*b*); *t*, mucilaginous thread holding the ball to the mother colony.

into the ball before it breaks away. These swim about for a time near the old colony and then may settle down on a near-by stem. Apparently these do not long survive the attacks of their enemies, for later I have seldom been able to find them, and I have never seen an isolated adult. By thus losing a few individuals in every generation the colonies would evidently continue to get smaller, for after the ball breaks away no new individuals are added to the colony. Such a decrease in numbers would undoubtedly take place were it not for the overlapping of the broods. One generation of eggs is laid so soon after the other that, in many cases, a large number of the second brood hatch in time to get into the forming ball. Occasionally, but not often,

two balls are formed at the same time, thus dividing the young colony.

The ball of young individuals is spherical in shape and is very regularly formed. Indeed, this is one of the very striking things about these interesting balls. The feet of the individuals are not attached together in an irregular mass, as one might suppose, but they are arranged so as to form a very regular sphere. It is difficult to ascertain just how the animals are held together in these balls, but it seems probable that they are glued by the adhesive substance from the foot gland. This is supported by the fact that when a young ball is killed and placed in alcohol it breaks up, as if the adhesive material had been dissolved. The animals when in this ball are always nervously jerking back and forth. The ball swims freely by means of the currents from the trochal discs. If, for any reason, more of the individuals turn their trochal discs in a certain direction, the whole colony will move in that direction. That the movement of the ball is due to the summation of currents is shown by mixing some india ink with the water. It is then seen that the ball always moves in the direction from whence the stronger current comes. The ball swims with a revolving motion but is nevertheless able to move with considerable precision in a certain direction. A most important characteristic of these young balls is the fact that they react positively to light. One always finds them on the side of the dish next to the window. If they are placed in a dish in the sunlight, the balls congregate on the side towards the sun. If this region is shaded they *at once* leave it and move towards a part more strongly lighted. A considerable number of experiments showed that this reaction is very marked and constant.

The time which the animals spend in this swimming ball before settling down to form a permanent colony varies considerably, depending primarily upon the illumination. So far as I have observed, the colonies usually hatch out during the morning hours, the young ball breaking away about noon or afterwards. In no case have I seen them, under natural conditions, form a colony until evening or after sundown. As long as the dish is lighted, even by diffused light, the young rotifers continue to swim back and forth along the lighted side of the dish, striving apparently

to get as near the source of light as possible. But if the ball is placed in artificial darkness they very soon begin to form a permanent colony.

The method of forming this permanent colony is interesting. When no longer influenced by the light the ball begins to move about the dish in an apparently aimless manner. If in this wandering it chances to come against some piece of water plant or other object many of the young rotifers turn their trochal discs towards this, thus checking the progress of the ball. This reaction is brought about by purely tactile stimuli. If a clean needle is placed in front of a swimming ball, the latter will stop and the young rotifers move their trochal discs along the needle. Whether they will settle down and establish a colony seems to depend chiefly on food conditions. If the stem possesses but little débris the ball may move along it for some distance, but in most cases finally swims away. If, however, the stem has more débris attached to it the young rotifers persist in their efforts to place their trochal discs on this, probably in order to get the food particles. In this way the ball is prevented from moving away. If the ball remains in one place for a time, one or two of the rotifers will be seen dragging themselves out of the ball and moving slowly along the stem. In this manner they move a short distance and then jerk back, then start out again, each time going a little farther. Soon others come out of the ball and begin moving about, until a large number are found moving up and down the stem (Fig. 2). In moving along the plant the animals extend their bodies with the long axis parallel to that of the stem. A few of the cilia of the trochal disc and a few of those in the posterior circle appear to touch the stem and in this manner the animals "crawl" along the plant, reminding one very much of a brood of young caterpillars. But it is probable that the water currents are here as at other times the most effective agents of locomotion. In these movements there is no revolution on the long axis. In advancing along the stem the animal usually shows great hesitation in going over portions which have not been traversed by some member of the colony. In the space over which several rotifers have travelled others move with little hesitation, but on coming to new territory they move forward a

short distance and then draw back, often repeating this several times. Thus each individual pushes some distance beyond the previous limit, but at last it usually turns around and moves back towards its fellows. This like certain observations previously mentioned seems to indicate that the adhesive thread which they always secrete as they move about, furnishes a guiding stimulus for the young rotifers. Sometimes an individual leaves the stem and swims a short distance from it, always attached by the thread.

By such movements the whole ball finally breaks up and the individuals are seen moving back and forth along the stem, some-

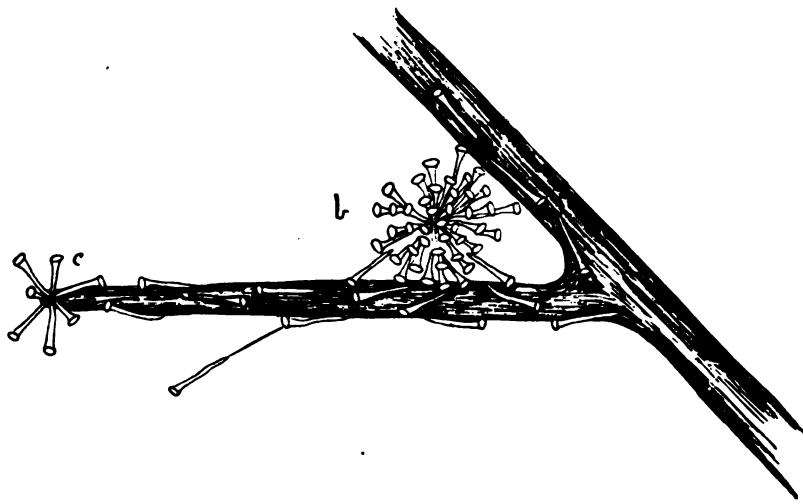

FIG. 2. Sketch showin the young ball (*b*) breaking up into free swimming individuals and the permanent colony forming at *c*.

times venturing quite a distance from the majority of the colony. But soon a few individuals settle down at some point and remain fastened by their posterior ends, with the body projecting at right angles from the stem (Fig. 2, *c*). Soon other individuals attach themselves in a similar manner with their posterior ends close to those already attached. This is the beginning of the formation of the permanent colony. In a comparatively short time all of the young animals have attached themselves in a closely aggregated group surrounding or nearly surrounding the stem. This permanent colony may be formed close to where the ball broke

up or at some distance from it. Sometimes it is formed at the end of a branch, sometimes at its base, or even on the main stalk; at other times in intermediate positions. If the swimming balls are allowed to remain in a dish containing no water plants soon after dark they begin to form a colony on the sides or bottom of the dish. Apparently when the stimulus from the light is removed the colony-forming instinct becomes dominant. When the young balls are placed in artificial darkness, colony-formation begins in from fifteen to thirty minutes and is usually completed within an hour or an hour and a half. Most of these colonies are found in well-lighted places. This doubtless arises from the fact that the swimming balls remain on the lighted side of the vessel and tend to form a permanent colony soon after this stimulus is removed. This is of advantage to the animals for they feed partly on diatoms and small protozoa which are more abundant in favorably lighted places.

Thus the young rotifers tend to remain attached in the swimming ball during their natatory life, but at the proper time they are able to sever this connection and to leave the ball one by one. Possibly the stimulus due to the contact of their posterior ends is sufficient to keep them together, until some stronger stimulus, such as food, overcomes the contact stimulus and induces them to separate. Hunger satisfied, they again respond to the stimulus of mutual contact and assemble anew, this time to form a permanent colony.

With the formation of this permanent colony changes take place both in the behavior and in the structure of the animals. Up to this time the young rotifers have behaved in a very nervous manner, constantly contracting and extending the body. But as soon as the animals settle down in the permanent colony they lose much of this irritability and remain with body and trochal disc expanded for long periods. Yet even in the adult colony one individual or another is frequently seen to suddenly fold the trochal disc and strongly contract the body, then at once begin to expand more slowly. Sometimes this contraction is evidently a reaction to some floating particle that has touched the trochal disc. More frequently there is no visible cause for the contraction. The animals in the permanent colony have another method

of reacting, not found or at least not so well marked, in the free swimming balls. When the colony is stimulated as by a slight jar to the dish or a disturbance in the water the whole colony contracts at once and if the stimulus is strong they may remain contracted for some time. This is the usual method of reacting to mechanical, chemical or electrical stimulation.

After the young animals have once formed a permanent colony they become incapable of repeating the colony formation. If some of the individuals of a permanent colony are removed from the stem they do not behave as they did previous to the formation of the colony. They swim about by means of the ciliary currents, but in an entirely aimless fashion. They neither attach themselves to the old colony nor form a separate colony. I have kept such removed individuals alive for several days but during this time they made no attempt to reform a colony.

There are likewise several structural changes which take place after the formation of the permanent colony. The animals soon show considerable increase in size, the trochal disc becomes broader and the notch on the ventral side of this makes its appearance. The small circlet of cilia at the foot of the animal disappears and the character of the cement secreted by the foot-gland changes. This cement is no longer dissolved by alcohol as it was in the young specimens. The gland is always much smaller in the adult than in the free swimming individual. But the most striking change and one which perhaps accounts for some of the changes in behavior, is the degeneration of the eye spots. As stated before, the rotifers when hatched possess two red eye spots; the reaction to light of the swimming ball is probably due to these structures. After the permanent colony has been formed for several hours one finds that the eye spots have disappeared from their previous position while two small red bodies are floating about in the body cavity. In most cases these bodies are inclosed in floating corpuscles which have considerable resemblance to leucocytes. Montgomery ('03) mentions the presence of certain non-cellular corpuscles floating in the body cavities of certain Floscullariidæ. He regards these as waste products. Since in *Megalotrocha* these bodies often enclose the degenerating eye spots and these latter are seen to

gradually waste away, it seems possible that they may function in the same manner as the leucocytes of many other animals. After the eye spots have disappeared from their original location the animals no longer react to light.

General and Comparative.

The main features in the formation of new colonies of this rotifer are then as follows: When first hatched from the eggs the young are free swimming, but do not leave the colony singly. These individuals come together into a swimming ball which reacts positively to light. Later under certain conditions this breaks up into free individuals again. These then aggregate themselves into a permanent colony in which the animals spend the remainder of their lives. In this colony formation the mucus-like secretion of the foot-gland plays an essential part.

The details of colony formation in other rotifers has apparently not been described, but it seems probable from the known facts that a similar sequence of processes occurs in some other cases. In *Lacinularia socialis* according to Huxley ('53), Hudson and Gosse ('89) and others, the process appears to resemble that in *Megalotrocha*. The young animals come together into swarms or balls and swim freely. Later permanent colonies are found on water plants. In two species of *Megalotrocha* from China, *viz.*, *M. semibullata* and *M. spinosa* described by Thorpe ('89 and '93), the adult colonies are free swimming. In these cases the eye spots are present in the adults. The new colonies swarm out of the old free swimming ones. Occasionally these balls may be found suspended from aquatic plants by mucilaginous threads. In the genus *Conochilus* we find swimming colonies of a slightly different nature. Here the free swimming balls consist of several adults with many of their young. According to Hudson and Gosse many of the newly hatched rotifers make a place for themselves in the adult ball by squeezing between the older members, while others of the new brood form new balls and swim away. There are several other more or less rare species of Melicertidæ in which free swimming or fixed colonies have been described. The free swimming adult colonies are interesting as apparently marking a step in the formation of the

fixed colonies. While the occurrence of colonies or swarms is common in the animal kingdom, I have seen no accounts of colony formation in other groups that resembles the processes seen in these rotifers.

BIOLOGICAL HALL, UNIVERSITY OF PENNSYLVANIA,
May 1, 1906.

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THE "ACCESSORY CHROMOSOME" IN EPEIRA.

EVIS HOWARD BERRY.

INTRODUCTION.

The problem involved in the following paper was suggested to me in August, 1905, by Professor E. B. Wilson, in view of the apparent contradiction that occurs between the insects and spiders in regard to the origin and behavior of the so-called "accessory chromosome."

In many of the Orthoptera (Acrididæ ('00), and Locustidæ ('02) McClung; Acrididæ ('00 and '02) Sutton; and Phasmidæ ('01) de Sinéty) the accessory chromosome has been found to be derived from a single, univalent spermatogonial chromosome, which splits longitudinally during the growth period, the two halves passing bodily to one pole in the first division. In the second division, however, these two halves were found to separate, being distributed equally to each of the resulting spermatids. Professor Wilson in his "Studies on Chromosomes," II. ('05), gives a general account of the accessory chromosome in the Hemiptera, which is consistent with the preceding results in the Orthoptera, though not identical. He says, p. 533: "The 'accessory' or heterotropic chromosome is certainly in most Hemiptera — and I believe will be found in all — unpaired in the spermatogonia, and its behavior is throughout that of a univalent body. . . . This chromosome divides in only one of the maturation divisions, passing undivided to one pole of the spindle in the other. The latter division is usually the second (*Pyrrochoris*, *Anasa*, *Protenor*, etc.), but in *Archimerus* and *Banasa* it is the first. In either case one half the spermatozoa receive one more chromosome than the other half."

Opposed to the foregoing conclusions are those of Montgomery ('05), obtained from a study of *Syrbula* (one of the Acrididæ), and of *Lycosa*, a spider. In these two unrelated forms he found the accessory chromosome of the growth period to be formed by the union of *two* univalent spermatogonial chromo-

somes at the time of synapsis. The mode of division of this bivalent accessory was not positively determined, but he suggested that the similarity in its formation gives some evidence that it may behave in the same way as the other chromosomes in both mitoses, dividing first reductionally and then equationally, thus being equally distributed to all of the spermatids. Nearly at the same time Wallace ('05) published her final paper on the spermatogenesis of *Agalena*, a spider, with results quite different from those of Montgomery on *Lycosa*. I will quote her brief summary, p. 182.

"1. The spermatogonia contain two accessory chromosomes and thirty-eight other chromosomes.

"2. In the primary spermatocytic division, the two accessory chromosomes pass over undivided into one of the daughter cells. The reduced number of other chromosomes is nineteen and these divide transversely.

"3. In the secondary spermatocytic division, the two accessory chromosomes again pass over undivided into one of the daughter cells. The nineteen other chromosomes divide longitudinally.

"4. Only one fourth of the spermatozoa contain the accessory chromosomes.

"5. Apparently the remaining three fourths of the spermatozoa degenerate after almost or altogether reaching maturity. In this respect they are regarded as homologous to the polar bodies thrown off by the ovum."

In view of these perplexing and contradictory accounts, I undertook the present work on *Epeira sclopetaria* to see if I could throw any light on the question. Owing to lack of material I have been able to study only one family, the Epeiridæ. The results which I have to offer on the origin and behavior of the accessory chromosome here are consistent with those of McClung, Sutton and Wilson, already mentioned, and also with those of Blackman on the myriapods ('03), but they give no explanation of the re-results of Wallace and Montgomery, which are widely different from my own. At a future time I hope to examine other families of spiders with respect to this discrepancy; certainly in none of my preparations of *Epeira* do I find any trace of degenerating spermatozoa.

I am greatly indebted to Professor Wilson for assistance in directing this work, and in the preparation of this paper. I wish also to thank Professor Calkins for his kindness in correcting the manuscript.

TERMINOLOGY, METHODS, ETC.

At Professor Wilson's suggestion I shall designate the "accessory chromosome" as the "odd chromosome," a name first used by Montgomery in a somewhat different sense.[1] Since there is no reason for calling it the "accessory chromosome," and since it behaves heterotropically in only one division, it seems advisable to adopt the simpler name, with the important significance that it has no mate throughout the history of the male germ cells.

Material was obtained in August and September, 1905, and also again in the early spring of 1906. The testes were dissected out rapidly in the fixing fluid, instead of the customary normal salt solution, as the process was thus rendered much easier. Strong Flemming gave the best fixation, and the finest results for general study were obtained with iron hæmatoxylin and pure saffranin, the latter giving especially beautiful and valuable results. Several different stains were tried as a differential test for a plasmosome, thionin, Auerbach and Flemming-triple. Long-extracted iron hæmatoxylin slides were studied also, but in no case could I find any trace of a plasmosome.

The figures for this paper are camera drawings made with a compensating ocular, No. 12, and a $\frac{1}{12}$ oil immersion lens. They were enlarged $2\frac{1}{2}$ diameters with a drawing camera, corrected from the original, and then reduced one half in the final plates.

SPERMATOGONIAL CHROMOSOMES.

Longitudinal sections of the testes give a complete series of stages from the resting spermatogonia around the periphery to the ripe spermatozoa in the central lumen. For this work it did not seem necessary to make a detailed study of the spermatogonia, the one important thing being to determine the number

[1] In his latest paper ('06) Montgomery recognizes the fact that there is no distinction between the "odd chromosome" and the accessory, as was pointed out by Wilson ('05). Montgomery now proposes the term "monosome" for this chromosome.

of spermatogonial chromosomes. With this in view I selected eight of the best polar metaphases of the last spermatogonial division, and drew them carefully with the camera. Seven of the

FIG. 1. *a–c*, last spermatogonial division; *a*, side-view of spindle; *b*, *c*, metaphase groups, showing twenty-three chromosomes, odd chromosome (*o*); *d–i* growth period, showing characteristic forms of the odd chromosome (*o*); *d*, contraction phase, early growth period; *e*, *f*, later stages showing the shortening and thickening of the spireme threads; *g*, *h*, late growth period, beginning of condensation; *i*, prophase of first division.

eight camera drawings gave a count of twenty-three chromosomes. In the eighth the chromosomes were so compact that I could not count definitely beyond nineteen, but from the seven

good ones it seemed conclusive that twenty-three was the correct number. Owing to the fact that the chromosomes often lie one upon the other, these counts were made with difficulty. In Fig. 1, *b* and *c*, distinct size and form differences may be readily observed. These variations are due in part to foreshortening, yet allowing for this error twenty-two of the chromosomes can be symmetrically paired off into eleven pairs, while the twenty-third is left without a mate. This is the small, round chromosome marked *O*. Since this chromosome has no mate, it can take no part in synapsis, and I infer that it is the odd chromosome, which persists as a compact, deeply-staining nucleolus throughout the growth period, Fig. 1, *d–i*.

GROWTH PERIOD.

Fig. 1, *d*, represents the contraction phase of the early synaptic period, with the ordinary chromosomes in the form of a closely massed spireme, while the odd chromosome appears as a large compact, deeply-staining nucleolus at one side of the contraction figure. Even as early as this the odd chromosome often appears double as in *f*, consisting of two closely approximated halves. This I interpret as the result of an equation-division. This double nature has been observed in other forms (*cf. Syromastes*, Gross; *Anasa*, Wilson; *Brachystola*, Sutton; Acrididæ, McClung). In the spiders Wallace and Montgomery have described the two accessory chromosomes as still separated at this period. In my own preparations I find a few cells, of the early growth-period, with two compact nucleoli, sometimes lying side by side and sometimes widely separated. At first I thought one of these might be a plasmosome, but with all of the differential stains mentioned above, both bodies took the nuclear stain equally well. These cells are of rare occurrence, and are, I think, abnormalities, resulting from this early equation-division of the odd chromosome.

In a later stage (Fig. 1, *e*) the chromosome threads loosen, and appear as longitudinally-split rods, with knobbed ends, the odd chromosome here being often bipartite in appearance. These chromosome threads shorten and thicken to form the chromosomes of the late growth period. In *g* and *h* the eleven ordinary

chromosomes have assumed the characteristic forms of rods, V's, rings, crosses, etc., preparatory to the final condensation of stage *i*.

Maturation Divisions.

In Fig. 1, *g* and *i*, the odd chromosome is seen in two of its most characteristic forms, which we encounter again and again

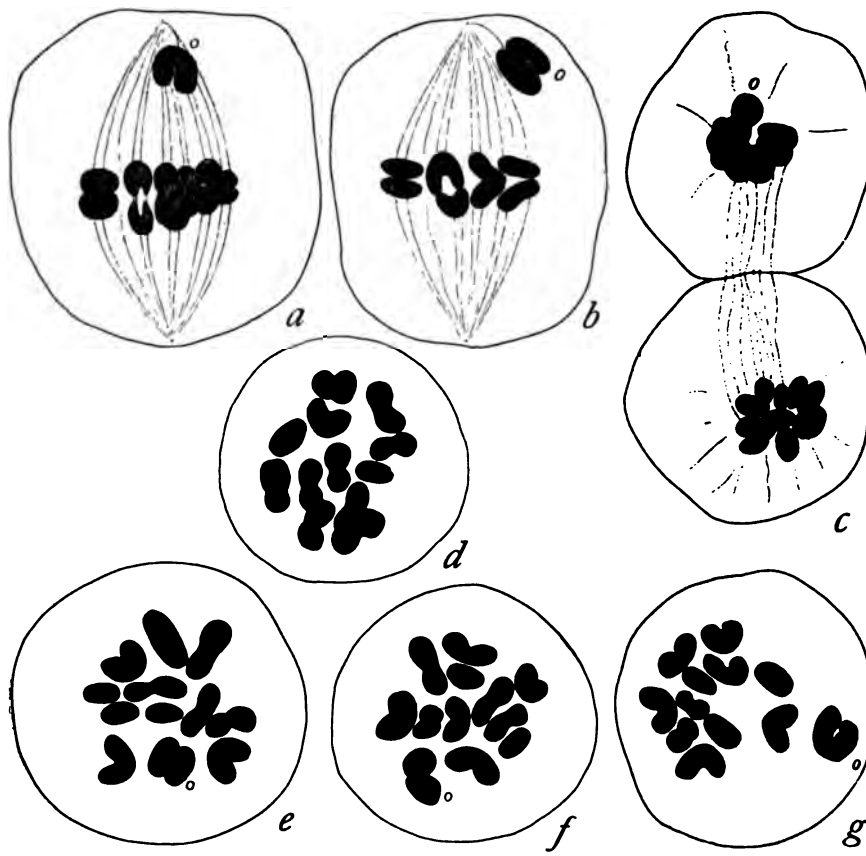

FIG. 2. First spermatocyte-division; *a*, *b*, metaphase-figures, side-view, showing odd chromosome passing undivided to one pole; *c*, telophase showing the odd chromosome present in but one of the daughter-cells; *d*, *e*, *f*, *g*, metaphase-figures, polar view, showing the odd chromosome, identified by its different level.

on the first division spindles. Fig. 2, *a* and *b*, represent such forms, attached by one spindle fiber and passing bodily to one pole in advance of the other chromosomes. These first division

figures are exceedingly numerous, the odd chromosome always being in this relation to the other chromosomes. From the similar shapes and positions of the ordinary chromosomes on the spindle, as compared with those described by other observers, I should say that the first division in *Epeira* is a reduction division and the second an equation-division, but I have not yet studied for this point and have no new evidence to offer.

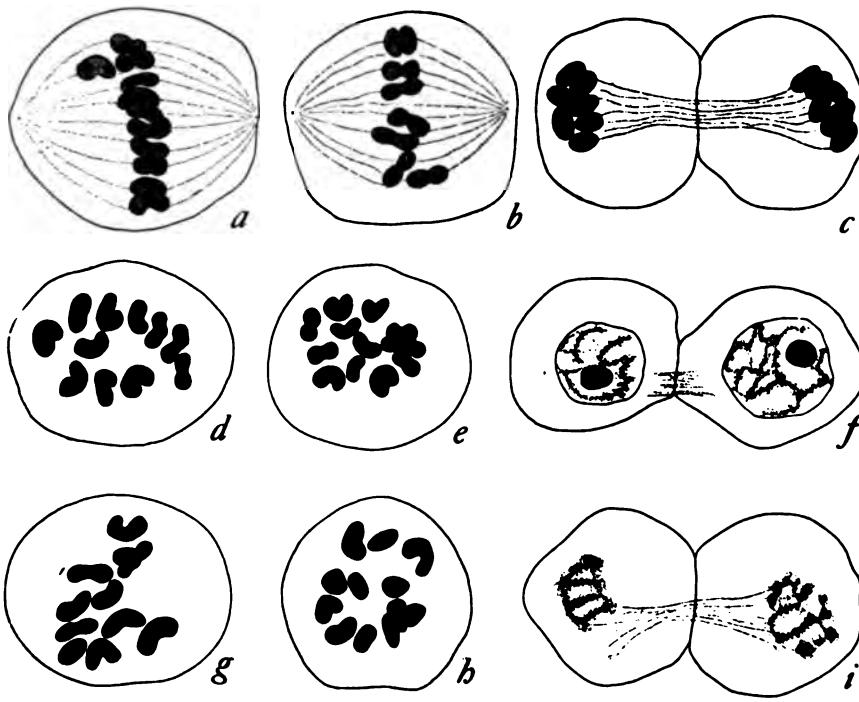

FIG. 3. Second spermatocyte-division; *a*, *b*, spindles, side-view, *c* — late anaphase; all of the chromosomes not pictured in *b* and *c*; *d*, *g*, metaphase-figures, showing eleven chromosomes; *e*, *h*, metaphase-figures, showing twelve chromosomes; *f*, *i*, telophases — *f* showing presence and equal distribution of odd chromosome, *i* showing absence of odd chromosome.

Fig. 2, *d–g*, represents first division polar metaphases showing the eleven ordinary chromosomes and the odd chromosome, *O*, which can always be recognized by its different level.

Fig. 2, *c*, represents a first division telophase, showing the odd chromosome in but one of the daughter cells (*o*, in the upper

half). In the lower cell the eleven ordinary chromosomes can be counted. Resulting from the division of each primary spermatocyte are two types of secondary spermatocytes, one containing twelve chromosomes, and one eleven. These two types divide, giving polar metaphase groups that show either twelve or eleven chromosomes (*cf.* Fig. 3, *d* and *g* with eleven, and *e* and *h* with twelve chromosomes).

In side view the second division spindles are all of exactly the same type (Fig. 3, *a* and *b*). I have studied a great many of them, and in no case do I find the odd chromosome passing undivided to one pole as Miss Wallace describes for *Agalena*.[1]

As I have suggested before, the second division is an equation-division, and the odd chromosome divides. in all probability, along its original split, thus being distributed equally to each of the resulting spermatids. The telophase, Fig. 3, *f*, represents two of the early spermatids derived from the preceding type of division, the odd chromosome still recognizable by its definite shape and deep-staining reaction. From the division of the other type of spermatocyte Fig. 3, *i* (without the odd chromosome), are derived two spermatids without this dark nucleolus. There are, therefore, two types of spermatids existing in equal numbers, one half with the odd chromosome, and one half without. To establish this point, I counted in forty-four different fields, all of the early spermatids with the odd chromosome, and all of those without. This count gave a total of 287 of the former and 286 of the latter, thus giving very positive evidence that the two types of spermatids exist in the proportion of half to half.

Summary.

1. The spermatogonia contain twenty-three chromosomes, twenty-two ordinary ones and the odd chromosome.

2. The odd chromosome is the chromosome-nucleolus of the growth period.

3. In the first maturation-division the odd-chromosome passes

[1] It seems possible that Miss Wallace may have mistaken a first division spindle for a second-division. Her two types of second-division spindles, Figs. 27 and 28, Plate IX., vary considerably in size, and by comparison of the larger, Fig. 28 (the one with the accessory chromosomes), with Fig. 18, it could, I think, easily represent a first division.

undivided to one pole, the eleven ordinary chromosomes dividing by a reduction-division.

4. The second maturation division is an equation division, in which the old chromosome participates.

5. Resulting from the first division are two types of secondary spermatocytes (1) containing eleven chromosomes, plus the odd chromosome, and (2) containing only eleven chromosomes.

Resulting from the second division are two types of spermatozoa (1) containing eleven chromosomes, plus the odd chromosome, and (2) containing only eleven chromosomes. These two types of spermatozoa exist in equal numbers.

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May 16, 1906.

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THE FORMATION OF PROGLOTTIDS IN CROSSOBOTHRIUM LACINIATUM (LINTON).

WINTERTON C. CURTIS.

I. Introduction and Summary.

The statement that all cestodes form their proglottids by the insertion of each new one between the scolex and the one last formed, has been current in our text-books for years and so far as I know there are no statements to indicate that any other mode of origin ever occurs. Upon turning to the original literature, one finds that the above statement rests upon the descriptions given about 1850 by Leuckart for the genus *Tænia* and closely related forms. This account was confirmed and supplemented by other authors about the same time, but in recent years we only find the process referred to as something established beyond all question as the universal method by which proglottids originate. No recent work so far as I am aware attempts to call this dogma in question, nor do writers longer think it worth while to record the fact that they have confirmed so well established a phenomenon.

Despite this universal acceptance our current description appears to be based upon published accounts which deal with a limited number of forms, though the fact that these accounts have not been contradicted leads one to believe that they describe the process correctly in those species which have been most frequently the subject of investigation.

The cestode, *Crossobothrium laciniatum*, a species occurring as an adult in the "sand shark," *Carcharias littoralis*, of our North Atlantic coast, does not conform to the current account in its method of forming proglottids. In this cestode there first appear from behind forward a considerable number of proglottids (Figs. 1, 2 and 10 of Pl. IV.). These "posterior" segments, as I shall call them, continue to form after the accepted fashion until about 35 of them have been produced. They are marked off by straight transverse lines and not until a considerably later period

do even the oldest of them begin to show the sculpturing of the posterior margin which is so characteristic for the adult.

When these posterior proglottids are about 35 in number, there begin to appear, in the unsegmented region just back of the scolex, others which may be termed the "anterior proglottids." These are produced in the reverse order, *i. e.*, the youngest is the most posterior one.

From such a condition as this (Fig. 10, Pl. IV.) the formation of proglottids continues so that from this time on we may say that the body is segmenting from both ends toward the middle. This process gradually obliterates the unsegmented zone (Fig. 12, Pl. V.) but specimens several centimeters in length may still show such a "zone" at a point near the line dividing the anterior and middle thirds of the body.

When this zone finally becomes obliterated there have been formed in the neighborhood of 50 "anterior" proglottids and upwards of 400 "posterior" ones. With this obliteration of the zone we reach what may be termed the fully formed adult condition as is shown by Fig. 14, *a–n. After such a stage is reached no more proglottids are formed.* and the specimen remains unchanged save for the continual maturing and separating off from the posterior end of the motile proglottids. This latter process finally reduces the total number of the segments to such an extent that the condition is perceptible at first glance. Such a specimen which had only 96 proglottids is shown in Fig. 16, *a* and *b*.

When the number of units in the strobilla has been reduced somewhat further, the region between the scolex and the first proglottid begins an active growth and elongates into a neck region as is shown by Figs. 21 and 15. After further elongation and the continual separation of the terminal proglottids, this neck region begins to form segments while there are still some of the most anterior segments of the old chain remaining (Figs. 17 and 24). This new segmentation begins as does that previously described by the appearance of posterior segments and later of anterior ones, and although I have no stages beyond the point shown in Fig. 24, I think one is justified in the opinion that this segmentation is the beginning of a new chain and that soon a new strobilla will be formed which would be with difficulty, if at

all, distinguishable from the one proceeding from the first segmentation. The specimen represented by Fig. 19, Pl. VI., is an exception to the above course of development, in that it shows all the characteristics of a specimen having the non-segmented zone of the early proglottid formation and at the same time an elongated neck. A possible explanation of this as a specimen undergoing regeneration after mutilation is given on p. 215 of this paper.

A discussion of the bearing these facts have upon our views regarding the nature of the cestode body in given on p. 217.

II. Discussion of the Literature.

In my examination of the literature I have relied upon Braun's monograph (Bronn's Thierreich) as a trustworthy summary of the facts established regarding the Cestoda down to about 1896, when the parts of this volume referring to the points in question appeared. Upon the particular subject of the method of proglottid formation the statements of this author are explicit in several instances and agree with the account which has long been current in our text-books. For example, in the introductory section on Cestoda (p. 1167), he says: "besitzen dem nach die Cestoden Zweierlei Theile: Scolex and Proglottiden: letzterer sind in einer Reihe dem Alter nacht geordnet, so das die jüngsten dem scolex am nächsten, die ältesten von diesem am weitesten entfernt sind." On p. 1224, under the paragraph entitled "Intercalation und Verwachsung von Proglottiden," Braun says: "wir haben allen Grund zu der Annahme dass die Proglottiden die ein Bandwurm besitzt oder jemals besessen hat, alle nach einander und zwar in der durch die Stellung gegebenen Reichenfolge am Scolex sich gibildet haben, dass also niemals durch einschalten neuer Proglottiden die Zahl der Glieder an einem Bandwurme vermehrt wird." Again, under the section of the Embryology entitled "Die Uebertragung der Finnen in den Endwirth," etc. (p. 1592 on), he refers in a number of places to the appearance of the segments, but makes only indirect mention of the order in which they appear, the evident reason for this being that there is only one method of proglottid formation known.

On page 1597, Kuchenmeister, von Siebold, Lewald, Haubner and Leuckart are quoted as having studied this stage of the development in a number of forms about 1850, since which very little attention has been paid to it. Leuckart's first work upon this point appeared in 1856 and was subsequently elaborated in the editions of his text-book.

On p. 1600, Braun says: "Die näheren Vorgänge bei der Bildung der Proglottiden sind uns bisher noch ganz unbekannt, nur die Anlage und Entwickelung der Genitalien ist bei manchen Arten erforscht."

From indirect references at other places in the volume it is clear that the accounts current in our text-books are justified by the absence of any published description contradicting the accepted method of proglottid formation.

The amount of literature published on Cestodes up to 1896 is enormous, but the manner of proglottid formation was established and had been explicitly described for so long a time before this date that one can hardly believe any marked exception to the universal method could have escaped the notice and comment of so thorough a reviewer as the author of this monograph has shown himself to be. Upon such a point as this I feel that Braun may be taken as reliable and that I am justified in the conclusion that no such process of proglottid formation was described previous to 1896.

I have consulted a considerable amount of the literature on Cestoda appearing since that date, at first hand, and have cov ered the abstracts and titles of cestode papers as they appear in the *Zoölogische Jahresbericht*, without finding anything to indicate that accounts have been published of a method of proglottid formation other than the one commonly accepted as the universal.

Papers upon other phases of the proglottid question have appeared, as for example one by Lühe, '98, who, in his examination of the segmentation of *Ligula*, found that the segments which occur at the anterior end do not correspond to the arrangement of the genitalia where these occur toward the posterior end of the segmented area and hence, that this case is not one of true proglottid formation but rather to be considered as a differentiation of another sort. While there may be some homology

between these pseudo-proglottids of *Ligula* and the "anterior" proglottids of *C. laciniatum*, I have made it clear that the greater part, if not all, of these structures appearing at the anterior end become true proglottids in the form I studied.

Instances of irregularities like the occurrence of half proglottids are noted by Braun, p. 1225, and dismissed as abnormalities. Such a condition has recently been discussed by Child ('02) for *Moniezia*, but this again has nothing to do with the points I have brought out for *C. laciniatum*. The fact that no exception to the typical method seems to have been recorded is to my mind justification for an extensive account of the mode of proglottid formation which I have observed in *C. laciniatum* and such an account is therefore given in the section which follows.

With the exception of the work by Linton, who described the species, I know of nothing which has been published upon *C. laciniatum*. This author in his original description (Linton, '86, p. 474, Pl. III.) speaks of small specimens and of the segments occurring at either end in somewhat larger ones. The posterior segments are, he says, "totally unlike those of the adult" and "are evidently evanescent." It is, therefore, likely that Linton had among his specimens the various stages which I have described, but he paid little attention to them and hence his brief paragraph does not bring out their significance.

The "long-necked" specimens were also observed by Linton and are referred to on several occasions. In his "Report of Parasites Collected in 1898" (Linton, '99) he records several instances, and in an earlier paper ('89, on pp. 800–801) and Pl. VII., Fig. 4, he describes the "long-necked" forms and others with a reduced number of segments in such a way as to make it clear that he has seen all the stages up to the point where the segmentation of the neck region begins. While not thinking that they justify the erection of a new species he speaks of them as a variety (longicolle) of *C. laciniatum* and suggests that they may represent the transition to another species. It is thus evident that Linton being interested primarily in the systematic work, did not examine this point sufficiently to ascertain the real significance of such stages. The importance of his observations for my work is that they confirm my statement that a certain pro-

portion of long-necked specimens are always found when any large number of the individuals of this species is examined. This is borne out by the observations of some of my colleagues on the staff of instruction at the Marine Biological Laboratory, who have frequently observed such individuals among the specimens used by the students. In this connection it may be said that all the specimens which have been examined by these men, by Linton and by myself have been taken between June 15 and September 15. Since the sand sharks are not present about Woods Hole from November to June (Smith, '97, p. 89) one could not obtain data throughout the year. It would, however, be desirable to examine the worms from sharks taken as late and as early as possible, for there may be seasonal relations such as obtain in the period of maturity in other forms,[1] and one might find at another period a relatively larger number of "long-necked" forms.

III. Detailed Account of the Proglottid Formation.

(*a*) *Material and Technique.* — The *C. laciniatum* from which the data here presented have been obtained were collected at Woods Hole, Massachusetts, during several summers (1902–'04). I have examined a large number of specimens which have accumulated since I began work upon this form and by selecting out the different stages have been able to demonstrate every step in the history of the strobilla with an abundance of material. In some cases, the specimens were killed under the pressure of a cover slip, but for the most part they were taken from lots which had been killed by placing for a moment in fresh water and immediately transferring to the killing fluid. All the material used in this study was fixed in saturated sublimate with 5 per cent. acetic. Almost any good stain will bring out the division lines between proglottids, but for determining the location of the reproductive organs in whole mounts Partsch's alum cochineal gave most excellent results.

(*b*) *Proglottid Formation in the Larval Worms.* — Out of an abundance of material, I have been able to demonstrate again and again that, in the case of young worms which have presum-

[1] See the remarks of Braun, p. 1461, upon the "time of reproduction," etc.

ably just entered the shark, the proglottids originate as follows: The young worm which is recognizable by the small size and the proportions of its scolex region (Fig. 1, Pl. IV.) at first forms segments from behind forward in the typical cestode fashion (Fig. 2, Pl. IV.). The youngest specimen I have found (Fig. 1, Pl. IV.) appears to have something missing from the posterior end, but a comparison with Fig. 2 which is a little older would indicate that not more than one proglottid has been lost. The progress of this early segmentation, the increase in size and the changes in the proportions of the scolex parts are seen by comparing Figs. 1, 2 and 5, which are all drawn on the same scale. The terminal proglottid in such stages is invariably long as in Figs. 2 and 4, but in older specimens (Fig. 7) which show no signs of having lost any segments at the posterior end one is very likely to find it much shorter. In Fig. 3, for example, there is a very long terminal proglottid which is bent twice in places which are clearly not lines of division between segments. Figs. 4 and 6 represent specimens in which the most posterior line of division was much fainter than the lines anterior to it. This suggested the possibility that the last two proglottids in such cases had originated by the division of an earlier single terminal proglottid. I have often found specimens which suggested very strongly that such a division of the earlier proglottids had taken place, but since it is well-nigh impossible to make certain of such a process when specimens can only be taken and arranged in series after being studied, I do not wish to say more regarding this than that such cases as shown above have frequently come to my attention and suggested the possibility of the subsequent division of the elongated segments found at the posterior end in very young worms. The formation of simple rectangular segments from behind forward progresses steadily (Figs. 6 and 7) until from 40 to 60 of these "posterior" proglottids have been developed. There then begin to appear in the region just behind the scolex other proglottids. These "anterior" proglottids are from the first different in shape from the ones at the posterior region and this difference is, as will be shown, continued into the adult. The most important fact about these "anterior" proglottids is that they are differentiated from in front backward. Fig. 10 shows a specimen in which a number

of these "anterior" proglottids have appeared, Fig. 7 one in which there are a few more and the differences between the proglottid outlines is more apparent. These two figures moreover illustrate the absence of a very definite relation between the number of posterior segments formed and the time at which the anterior ones begin to appear, for in Fig. 7, there are 56 posterior and 10 anterior as against 66 posterior and in 6 anterior in Fig. 10. The marked differences in contour are better seen a little later, when there are more anterior segments present. Fig. 8 illustrates this and shows that the oldest ones have already assumed the shape so very characteristic for the adult proglottids of this region, and even in those which are just appearing (Fig. 8) the suggestion of the four lappets which occur on the posterior margin can already be seen. The contour of segments from this region is seen again in the figures of Pl. VI. and a stage which is practically the adult is shown in Fig. 25, *a*, Pl. VII. The four lappets are very mobile in the living specimen and their appearance differs considerably according to their state of contraction and the angle from which the strobilla is viewed as will be apparent from a glance at Figs. 14, *a*, Pl. V., 16, *a*, Pl. VI. and 25, *a*, Pl. VII.

The "posterior" segments have in their final condition four flattened lappets (Figs. 14, *n*, Pl. V., and 25, *c*, Pl. VII.). This sculpturing is distinctly different from that of the "anterior" segments until the latter come to occupy the terminal position (Fig. 15, Pl. VI.), when they assume the contours of typical posterior proglottids.

The formation of the posterior segments continues rapidly — (Figs. 10, Pl. IV., and 12, Pl. V.) and this region presents an appearance identical with that ordinarily met with in the cestode strobilla. The anterior proglottids develop more slowly and their total number is always much less than the posterior ones, hence, the region where the two meet and where the non-segmented zone is finally obliterated is well towards the anterior end.

The total number of posterior segments can be determined only approximately because there is always the chance that some have been lost either under natural conditions or during the handling incident to collection and preservation. Taking the maximum

number in the longest specimens it appears that there may be formed upwards of 400.

In the case of the anterior proglottids the total number formed can be ascertained with more certainty for it is only necessary to count them in specimens where the zone is just being obliterated. Such counts show that there may be as many as 604 proglottids formed in the anterior region. I have found a few cases in which the zone seemed about to be obliterated when there were only 30–35 anterior segments, but in the great majority of such cases their number runs well up toward 60 and I think 50 + would be a fair statement of the average number.

The transition of the posterior segments into the unsegmented zone is always gradual, as will be seen in Figs. 13, Pl. V., 23 and 25, *b*, Pl. VII. On the other hand the anterior segments may show a gradual transition into the zone (Fig. 13, Pl. V.) or the transition may be more or less abrupt (Figs. 23 and 25, *b*, Pl. VII.) The condition shown by Fig. 25, *b*, is the more common one for the anterior proglottids while that shown in Fig. 13, Pl. V., is typical for the transition of the posterior ones into the zone.

When the zone is finally obliterated it is no longer possible to tell where it was located for the proglottids of the anterior end, with their four-pointed lappets, gradually change into the straight transverse lines which separate the many immature proglottids in the middle region of the chain. The adult condition thus obtained is represented by Figs. 14, *a–n*, in which typical regions of the body have been drawn and the length of the chain between such regions shown by straight lines. The peculiar anterior proglottids (Fig. 14, *a*) still show their characteristic outlines as far back as the 110th proglottid from the scolex (Fig. 14, *c*) from which we may conclude either that the specimen figured had formed a greater number of anterior proglottids than I estimated as the average, or that some of the proglottids formed behind the non-segmented zone have assumed the character of immature anterior proglottids. From the condition shown at the 110th segment of Fig. 14, *c*, we reach after a distance represented by the line *d* a region of very close-set immature proglottids which are separated by straight transverse lines without any sculpturing

of the margins. This condition is continued for a distance shown by the line *f*, before the rudiments of the reproductive organs are clearly discernible. When a place is reached where these can be definitely made out (Fig. 14, *g*) it is seen that the proglottids are beginning to assume the outlines characteristic for the posterior members of the chain. In *g* of Fig. 14, we find the reproductive organs well marked out at the region just in front of the 386th proglottid. They are first apparent in the whole mount at about the region of the 325th proglottid. From the region shown by *g* of Fig. 14 to the posterior end the transition along *h* and *i* to the 471st at *j* is gradual and from this latter point through *k*, *l* and *m*, to the terminal region, shown by *n* of Fig. 14, the change consists more in the elongation of the proglottid than in its growth in bulk. The 506th proglottid which terminates the chain shows fully developed reproductive organs and some eggs accumulated in the uterus and is farther advanced than many of the free motile proglottids one finds in the intestine.

(*c*) *The Reduction of the Primary Strobilla and the Formation of the "Long-necked" Stage.* — Such a specimen as the one just described may be termed *a young adult*, for it still has about the number of proglottids which we have reason to believe is not far below the maximum and it is already liberating ripe segments from the posterior end. The following points are of importance in this connection: First, the reproductive organs do not begin until well back of the 300th proglottid. Though sections might show them being laid down in front of this region, there is in any case a long region extending beyond the anterior half of the worm in which they have not yet appeared. Second, the non-segmented zone has been obliterated and the place where it disappeared can no longer be recognized. Third, there is no point in the chain where there is anything to suggest the interpolation of new segments. I have examined a large number of specimens in this and in the stages next succeeding without finding the slightest indication that new proglottids are added in the region back of the solex or elsewhere.

The only change which takes place in such a specimen is the continual dropping off of ripe proglottids from the posterior end and the steady advance toward the scolex of the region of ap-

pearing reproductive organs. Specimens are found in all stages intermediate between Fig. 14 and those which like Fig. 16 have a much smaller number of segments. It thus seems clear that such a specimen as is shown by Fig. 16, *a*, *b*, is to be regarded as derived from the "young adult condition" of Fig. 14 by the progressive loss of ripe proglottids and the advance anteriorly of the differentiating reproductive organs. In the specimen just cited (Fig. 16, *a*, *b*) there is a total of only 96 proglottids in the chain and the terminal ones are ready to be set free. The reproductive organs which in the whole mount are recognizable in the 26th proglottid have advanced beyond the point (30th) which we have fixed as the very minimum for the number of "anterior proglottids." There is as yet no sign of the appearance of new proglottids to make good those which have been shed. We might refer to this as an adult which is approaching the old age period of the strobilla in contrast to such a "young adult" as Fig. 14 shows.

Specimens with a smaller number of proglottids and showing the posterior ones well matured have been frequently observed, but when the number has been reduced much beyond this point (Fig. 16) one finds an important change occurring in the region between the first of the old "anterior proglottids" and the scolex. This change consists in the growth of this region into what I shall call a "neck" which soon separates the scolex and the first proglottid by a very appreciable distance (Fig. 21, Pl. VII.). In this figure the reproductive organs are recognizable in the 17th proglottid and probably occurred further forward but the specimen from which this was drawn was poorly stained for this point. Such a specimen as Fig. 15, Pl. VI., shows the reproductive organs well established in the 5th segment while in the 10th and last one they are fully matured. Such a case as Fig. 18, Pl. VI., has clearly come from an old chain which has formed a "neck" to which two of the old anterior proglottids are attached. It is very possible, however, that this specimen which I found among many other preserved specimens has had some segments detached in the collection or subsequent handling.

Specimens in which the neck has become well established next show a segmentation at the posterior end of the neck region (Fig.

17, VI., and 22, VII.), and this segmentation progresses from behind forward as did the formation of "posterior segments" previously described. When from six to twelve such segments have been established there begins a segmentation of the front end of the neck. This proceeds from in front backwards as did the formation of "anterior" proglottids at an earlier stage. Specimens showing the above changes are shown in Figs. 17, Pl. VI., 22, Pl. VII., 20, Pl. VI., and 24, Pl. VII. The number of proglottids of the old chain which are present when the neck begins to appear and later the number present when the segmentation of the neck begins, appears subject to a considerable variation. For example, Fig. 15, Pl. VI., and Fig. 21, Pl. VII., are specimens which when the state of contraction in each case is considered appear to have the neck developed to about the same extent, but the number of remaining proglottids is ten in one case and thirty-four in the other. Nothing can be determined on this point from Figs. 17 and 18, Pl. VI., or 22 and 24 of Plate VII., for although Figs. 18, 22 and 24 had the number of proglottids indicated when they were taken from the shark they may have been mutilated when the valve was cut open, while in the case of Fig. 17 I have no record of the condition when collected and hence suspect the possibility that some segments may have been detached by accident. Again, in Fig. 20, Pl. VI., is shown a specimen in which the neck has both anterior and posterior segments though forty-nine of the old proglottids remain. This is the maximum number of the old segments of which I have ever found upon a necked specimen. It would seem, therefore, that the neck region appears when the supply of proglottids comprising the earlier chain has become so much reduced that the developing reproductive organs begin to appear well into the region occupied by proglottids which must have had an "anterior" origin in the embryo, but the number of primary proglottids remaining when the neck appears is quite inconstant.

In the early part of my work, when I had not obtained so complete a record of the conditions in the "necked" specimens, I was inclined to question whether the segments formed at the anterior end in the larval stage (Fig. 8, Pl. IV.) were to be regarded as proglottids in the same sense as those developed posteriorly,

i. e., whether we had not to do with what one might term pseudo-proglottids as distinguished from the true or posterior proglottids. This led me to consider the criterion by which one would determine whether a region of the body was a proglottid or not. My view is that we are justified in applying the name proglottid to any region of the body which is set off from the neighboring parts by constrictions and which contains a complete set of reproductive organs. From many specimens which were examined when the non-segmented zone was almost obliterated, I found as previously stated that no specimens observed showed less than 30 + segments of "anterior" origin and that the number produced in this region was 50 + in the majority of cases. If this is so, specimens like Figs. 15, Pl. VI., and 21, Pl. VII., show us that such "anterior" proglottids may eventually become sexually mature and be shed off the same as any other in the chain. Moreover, as will be seen in the two figures just cited, anterior proglottids thus becoming ripe change their shape by the flattening of the four-pointed lappets of the earlier stage and assume exactly the shape characteristic for a ripe proglottid of posterior origin. I think, therefore, that we may dispose of any question as to whether the segments originating from in front backwards are to be considered as true proglottids.

The new segmentation appearing at either end of the neck region is identical with that which occurs in the young worm save that the "anterior" proglottids begin to appear before so many of the posterior ones have been developed. Were it not for the single terminal segment and for the slightly greater size of the bothria on the scolex one would certainly class specimens like Figs. 22 and 24, Pl. VII., as young worms similar to those shown in Figs. 7 and 10, Pl. IV., and 11, of Pl. V. The size of the bothria would not be very reliable in preserved specimens, but the single terminal proglottid with its four-pointed lappets would seem unquestionably to be the first anterior proglottid of a preceding strobilla.

I have not been able to secure stages showing that the segmentation of the neck region finally results in a new strobilla. The difficulty in distinguishing specimens, which have passed farther along the way to this condition than Fig. 24, from stages in

the primary strobilla would be considerable unless one of the old anterior proglottids still remained and I have not felt that the prospect of finding such stages justified my delaying the completion of this paper until I had another opportunity to examine a large amount of fresh material in search of them. This segmentation of the neck region is either the beginning of a new strobilla which to distinguish from the earlier we might call the secondary strobilla, or it is simply an abortive attempt in the old age of the individual and comes to naught. From what we know of the longevity and continued production of proglottids in a few other cestodes, Braun, p. 1604, I think the presumption is distinctly in favor of the former view, *viz.*, that the segmentation which I have observed beginning in the neck region is the first step in the formation of a new strobilla. Though it would be almost impossible to demonstrate the formation of other strobillæ from new neck regions after the secondary one has in turn become exhausted, we may, I think, consider such a process as not at all unlikely. Hence, we have the suggestion that what may be called primary, secondary and tertiary, etc., strobillæ are successfully headed off by a single individual scolex.

(*d*) *An Exceptional Condition, Perhaps to be Explained as Regeneration after Chance Mutilation.* — Two specimens have come under my observation which apparently do not find any place in the above scheme of development. One of these is shown in Fig. 19 of Pl. VI. In this we have a body with a definite zone region and the anterior and posterior segments which characterize a worm developing the primary strobilla. In contradiction to these features, we find a well-developed neck region. The size of the bothria gives no trustworthy evidence of the age in these specimens. I think these two exceptions may be explained as cases of regeneration after a chance mutilation occurring under natural conditions.

It will be noted if Fig. 19, Pl. VI., is compared with Fig. 12 of Pl. V., that the number and development of the anterior proglottids is relatively much greater in Fig. 19 than we find in the normal development of the primary strobilla. If such a specimen as Fig. 11 or Fig. 12, Pl. V., had been cut in two somewhere near the forward region of the posterior proglottids and

by this loss of a considerable portion of the young strobilla, the part having the scolex had been stimulated to form a neck for the production of more segments, we should have such a condition as Fig. 19 shows. It is commonly assumed in the theories regarding the nature of the cestode body that the Cestoda have great powers of regeneration. For example, Lang ('88) used this supposed capacity for regeneration as the starting point in his explanation of how the strobillization was first introduced into cestode phylogeny. We have, however, little in the way of direct evidence either from observation of specimens regenerating under natural conditions or from accounts of their regeneration under experimental control. The difficulties of technique have so far as I know prevented any experimental confirmation of this supposed power of regeneration. Still the assumption seems reasonable and the difficulties could doubtless be overcome if it seemed worth while to determine what regenerative powers the Cestoda do actually possess. In the case of *C. laciniatum* one should be able to test the explanation I have just given for these exceptional cases by the mutilation of young specimens in the way indicated. If one could overcome the difficulties and keep the worms alive by introducing them into another shark or otherwise, I think some very interesting data might be obtained by the mutilation of strobillæ in different stages and a study of the regeneration if such a process occurs.

(*e*) *Histology of the Non-Segmented Zone in Young Worms and of the Neck Region in Older Specimens.* — I have examined sections of this region which is the place where differentiation appears to be most active hoping to find some characteristic features by which its histology might be distinguished from that of the ordinary proglottid, but with the material available have not been able to recognize any such distinguishing features. If nuclear division is in progress it must be amitotic such as Child ('04) has recently described for *Moniezia*, for the material I have would show clearly the existence of mitotic figures. I have endeavored by staining after the method followed by Child to determine the presence or absence of such division but the fixation is not quite good enough for this point. This histology seems to be much the same as that of the formed proglottid save that

the muscle fibers are less developed. In the young worm the zone has a circular outline in section while in the neck region it is somewhat flattened. Fig. 26 which represents the structures found in this zone of a young worm shows that there occur only the structures which are regarded as typical for the adult cestode (Schneider, '02, p. 311, Fig. 324, and Braun, Pl. XLVII.). There are circular and longitudinal muscle fibers between the cuticle and the palisade of subcuticular cells, the latter being grouped so that they form lines between the outer portions of these cells. The parenchyma contains scattered nuclei, some with cytoplasm aggregated about them as in a definite cell body. One member of each pair of water tubes appears in the figure. The results of this examination are disappointing, for unless there is an active amitotic division, which I fail to make out with this material, there is nothing which would suggest the active differentiation which is taking place. However, I do not offer this as in any way an adequate discussion of the histology of this region. Such an account I hope to give at some future time when I have proper material for a thorough examination. This is merely a statement of what I have been able to accomplish along this line thus far.

IV. General Considerations.

If my interpretation of the stages found is correct, we have in *Crossobothrium laciniatum* a method of proglottid formation which is radically different from the one now accepted as universal. Also, there seems to be more than one strobilla formed so that we may speak of primary and secondary strobillæ and perhaps there are even more. This is in marked contrast with such forms as the tænias which present a continuous growth and differentiation in the neck region to make good a continuous shedding of ripe proglottids. If we try to analyze the proglottid formation here described and to compare it with the process which occurs in other cestodes, we may do so in the following way. The appearance of the first proglottids at the posterior end is homologous to the method now accepted as the universal one. Such a stage as is shown by Fig. 5, Pl. IV., and from this on to the time when the "anterior" proglottids begin to appear, may

be regarded as a cestode with an unusually long neck region. The essential point of difference comes when proglottids begin to differentiate at the anterior end of this neck *in the reverse direction.* It must however be noted that subsequently the front part of this neck region becomes the neck of the next strobilla which at its posterior end differentiates from behind forward. It is thus possible to regard the segment formation of *C. laciniatum* as derived from the ordinary type by the acquisition of a long neck and subsequently its differentiation in the reverse order at the front end.

While it is clear that the proglottids of the adult are serially homologous throughout the worm, we may ask ourselves whether there can be found any good reason for the modification of structure which is so noticeable in those of the anterior region. If we consider the case of such a "young adult" specimen as that shown by Fig. 14, *a–n*, Pl. V., it is clear that this modification of the four posterior corners into sharp projections is not a characteristic belonging exclusively to the proglottids which had an anterior origin. In this figure if we examine *a*, *b* and *c* we find that the area occupied by proglottids having the shape in question extends considerably beyond the region occupied by the proglottids which probably had an anterior origin. In such a specimen we can no longer determine where the non-segmented zone finally disappeared, but as has been pointed out there are never many more than 60 anterior ones and hence the zone must have been not far from the 60th segment. The proglottids having the shape in question are found extending even back of the 110th as shown in *c* of Fig. 14. This means when applied to such a figure as 13 of Pl. V., or 23 of Pl. VII., that some of the forward members among the simple proglottids behind the "zone" are later made over into the anterior type, and in Fig. 25, *b*, Pl. VII., we see that this may begin even before the zone is obliterated. It is thus clear that the fact of a proglottid having an "anterior" origin is not the factor which has determined the peculiar shape of the segments at the front end of an adult *C. laciniatum*, but that the antero-posterior differentiation which exists extends beyond the region of the obliterated "zone" and into that of the posterior segments. The cause would then seem

to be something which affected the anterior end of the strobilla independently of the origin of the proglottids involved.

It has occurred to me in collecting the worms from the spiral valve that perhaps the four projections which characterized these forward segments of the adult strobilla, may be regarded as an adaptation by which the worm is better able to retain its hold with the scolex. The passage along between the folds of the semi-solid feces which are often found in the valve, must often draw out the bodies of these cestodes and tax the hold of the scolex to a corresponding degree. The projections in question would be very effective in preventing the front part of the worm from slipping backward and would thus relieve the strain on the scolex. I believe that we must look to something in the conditions which act upon the anterior region of the strobilla and not to the anterior or posterior origin of any given proglottid, to account for this feature of *C. laciniatum* and the above suggestion is given as a possible explanation.

Since the theories regarding the nature of the cestode body have taken into consideration the supposed universal method of proglottid formation we may consider what bearing the facts here established have upon such theories. At the outset I will say that I do not think this single case of a small number of true proglottids developed anteriorly and in the reverse direction will justify any sweeping modification of existing views, for it is only fair to suppose that the posterior proglottids of *C. laciniatum* are strictly homologous to the entire strobilla in other cestodes and we may regard *C. laciniatum* as a species in which another method of proglottid formation has been superposed on an older and more universal one. The history of our theories regarding the nature of the cestode body is extensively summarized by Braun in the Cestode volume of Bronn's Thierreich. Beginning with the earlier conception of the chain as composed of proglottids which had become united, he traces the history of the theory developed by J. P. van Beneden, Siebold, Leuckart and others, which viewed the Cestode at a "polyzoötic" organism, *i. e.*, that despite the physiological unity of the chain of proglottids we are dealing, from the morphological standpoint, not with a unity but with an animal stock. This view has been main-

tained by some to the present day, having been defended more recently by J. v. Kennel in his "Lehrbuch der Zoölogie." On the other hand it has been subject to considerable attack which is summarized in the theory accepted by Lang and Korschelt and Heider. The latter authors hold that the scolex and a single proglottid represent the individual and are equal to the body of a trematode and that the present organization of the cestode body has been attained by the reduplication of that region which contains the reproductive organs. In a way this opinion is not so different from the other view since we may think of this reduplication as a budding process in which only the posterior half of the individual is formed.

Broadly speaking, the discussion reduces itself to the question whether we shall consider the segmented condition of the cestode as having arisen by the loss of individuality and the adherence together of originally complete individuals which were formed by budding, or as arisen by the elongation and reduplication of certain parts in a single individual. It is like the question of metamerism which though most commonly explained in accordance with the latter (Lang, '82, and others) has nevertheless been accounted for by some (Whitman, '99) along the line of the former view. Personally, I agree entirely with Braun in the concluding paragraph of the discussion above cited, that the theory one will incline to is largely a question of temperament since the phenomena can be interpreted in either way. The facts brought out for *C. laciniatum*, while they show a new method of proglottid formation do not turn the scale in favor of either interpretation. If such a condition as is here described could be shown to be more widespread in the cestoda and thus the one we now regard as primitive should lose some of its importance we might then believe that a segmentation proceeding from both ends toward the middle is better explained upon the hypothesis that reduplication of parts has occurred in a single individual than upon the theory that it has arisen through a process of strobillization identical with that by which the ephyræ of a scyphozoan are formed. But the facts regarding *C. laciniatum* stand alone and we can at present claim no more than that the formation of proglottids in the cestoda deserves renewed investi-

gation particularly in any forms which suggest a departure from the type of development hitherto supposed to be without exception.

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PLATE IV.

Figures showing stages in the proglottid formation of *C. laciniatum* as found in the spiral valve of the shark, *Carcharias littoralis*. All from camera lucida outlines. The magnification is given for the figures as reproduced.

FIG. 1. The youngest specimen obtained. The outline at the posterior end indicates that at least *one* proglottid has been lost, but the small size of the scolex shows that this one is slightly younger than the complete specimen shown in Fig. 2 and hence it is unlikely that more than one proglottid is missing. × about 50 diameters.

FIG. 2. A complete specimen slightly older than that of Fig. 1. The first proglottids are forming from behind forwards in typical cestode fashion. × about 50 diameters.

FIG. 3. The posterior region of a specimen in about the stage of Fig. 2, showing a very long terminal proglottid which is bent in two places, but does not show any lines of division. Compressed when killed and hence apparently wider than Fig. 2. × 18 diameters.

FIG. 4. A similar specimen showing a condition which suggests that a long terminal proglottid of an earlier stage has begun to divide into two. × 18 diameters.

FIG. 5. A specimen slightly older than Fig. 2. The scale of 50 diameters being the same as that of Figs. 1 and 2 shows the marked increase in size. × 50 diameters.

FIG. 6. Posterior portion of a specimen somewhat older than Fig. 5. To show typical dimensions of proglottids at this stage. The last division line is faint as though a longer terminal proglottid had just divided. × 18 diameters.

FIG. 7. An entire specimen in which the "anterior proglottids" are appearing. This is actually much larger than the specimen shown in Fig. 5. × 18 diameters.

FIG. 8. The anterior segments of a specimen which still showed an extensive non-segmented zone. × 42 diameters.

FIG. 9. The last six segments from the posterior end of a specimen similar to the one used for Fig. 8. × 42 diameters.

FIG. 10. A specimen of about the same stage as Fig. 7, but with fewer "anterior" and more "posterior" proglottids. A deeper constriction is noticed between the eighth and ninth proglottids from the posterior end. × 18 diameters.

FIG. 1

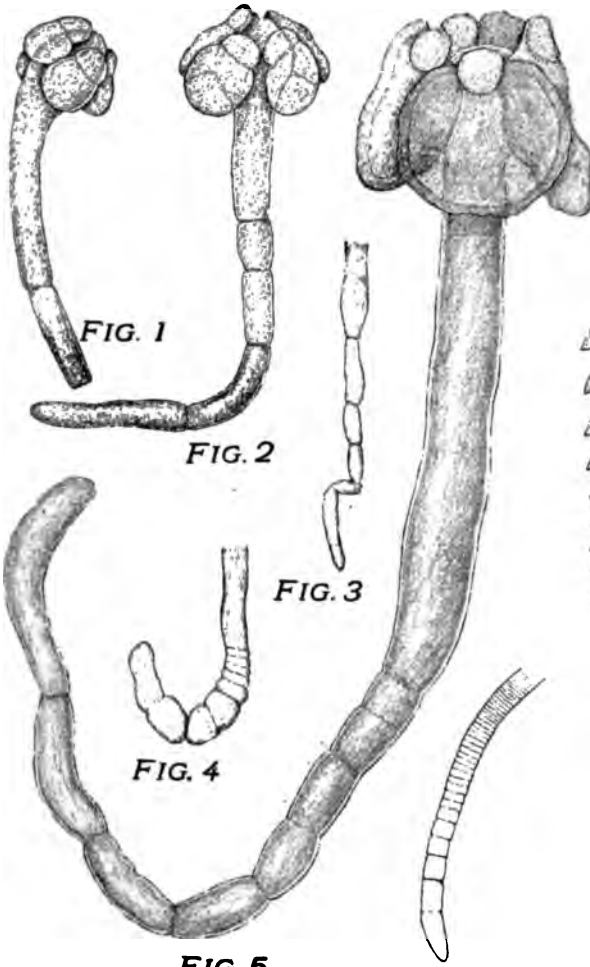

FIG. 2

FIG. 3

FIG. 4

FIG. 5

FIG. 6

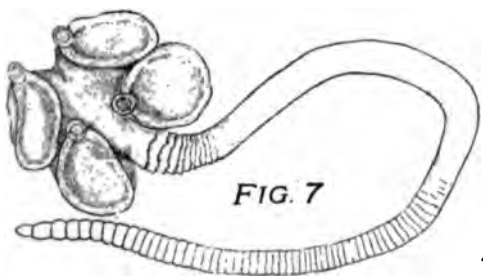

FIG. 7

FIG. 8

FIG. 9

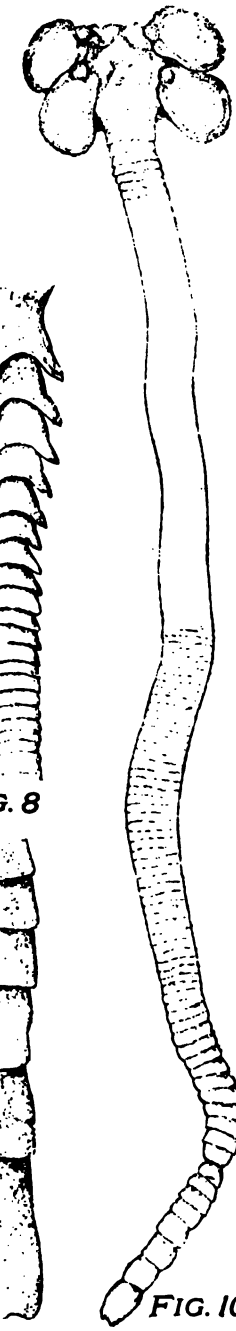

FIG. 10

PLATE V.

Figures of late stages in the proglottid formation of *C. laciniatum*. All from camera lucida drawings. The magnifications are for the figures as reproduced.

FIG. 11. A specimen with 8 "anterior" and 35 + "posterior" proglottids and showing a greatly elongated, non-segmented zone. × 12 diameters.

FIG. 12. An entire specimen in which the non-segmented zone has been greatly reduced by the appearance of 30 + "anterior" and 200 + "posterior" proglottids. × 12 diameters.

FIG. 13. The non-segmented region of a specimen in which the "anterior" and "posterior" segments are just meeting to obliterate the non-segmented area. Such a stage would be slightly beyond that of Fig. 12. In this instance there were 40 + "anterior," 250 + "posterior" proglottids. × 50 diameters.

FIG. 14, *a–n*, represents the appearance of the proglottids and their proportions in a young adult specimen which contains about its maximum number and is just beginning to liberate ripe proglottids at its posterior end. The length of the regions not drawn in is represented by the straight lines. The letters show the sequence in which the pieces should be arranged. The number of proglottids up to that point is marked at the posterior end of each section drawn. The total number of proglottids was about 506. × 12 diameters.

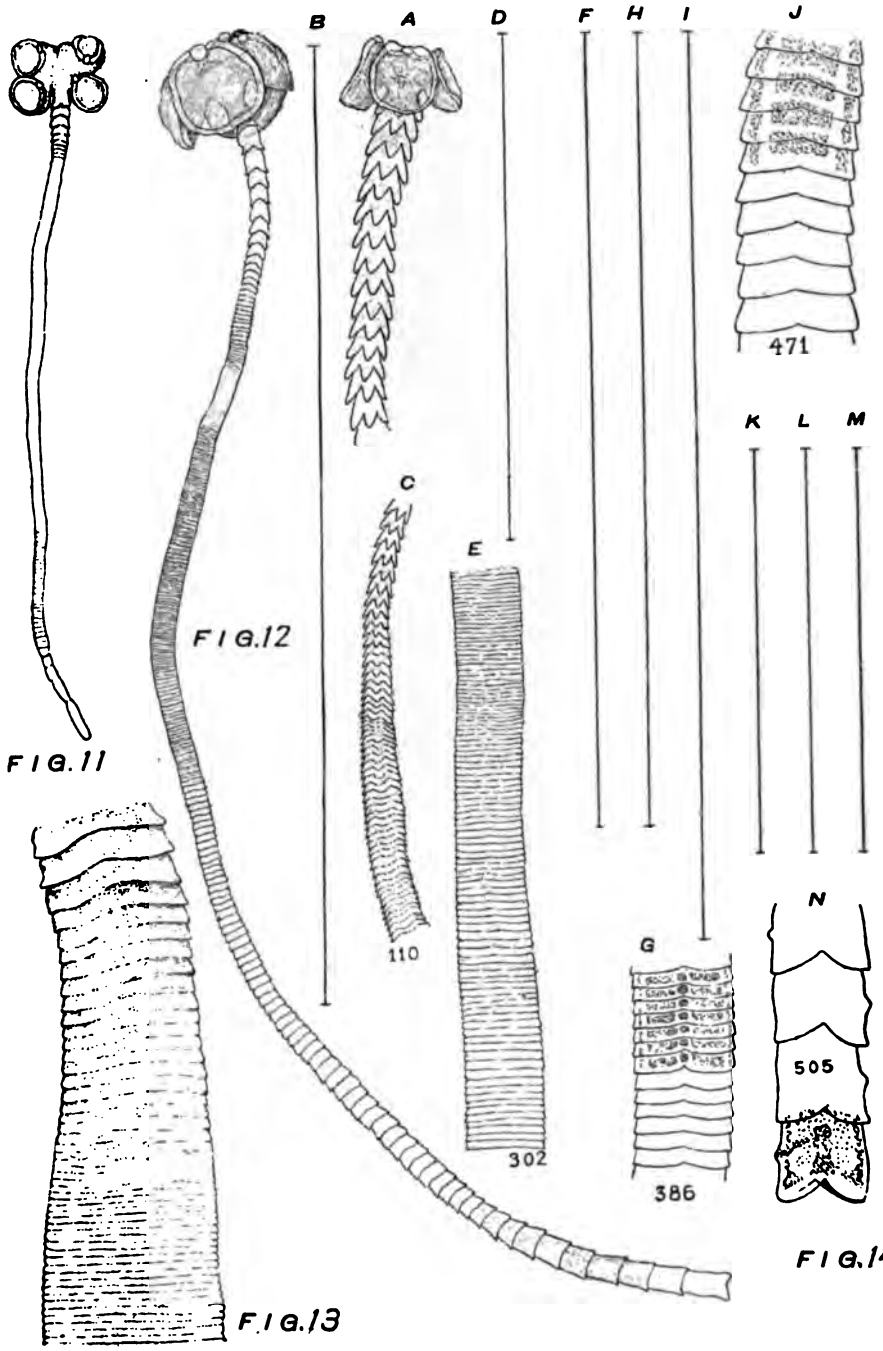

FIG. 11

FIG. 12

FIG. 13

FIG. 14

PLATE VI.

Figures showing the development of the "long-necked" specimens of *C. laciniatum*. All from camera lucida outlines. The magnification is given for the figures as reproduced.

FIG. 15. A "long-necked" specimen in which the reproductive organs can be made out as far forward as the fifth proglottid. The tenth and last is about ready to be shed. × 12 diameters.

FIG. 16, *a* and *b*. The entire length of a specimen which is approaching the "long-necked" condition. There is a total of 98 segments and the reproductive organs can be distinguished as far forward as the twenty-sixth proglottid. × 12 diameters.

FIG. 17. A "long-necked" specimen with 8 of the old anterior proglottids and the new segmentation begun in the posterior region of the neck. × 12 diameters.

FIG. 18. A "long-necked" specimen in which the new segmentation has not yet begun. × 12 diameters.

FIG. 19. A "long-necked" specimen with an unsegmented zone further back. See text for a possible explanation of this specimen. × 12 diameters.

FIG. 20. A "long-necked" specimen which has still 49 of the old proglottids in which reproductive organs can be made out as far forward as the third or fourth from the anterior end. The neck region has begun to segment at either end. × 12 diameters.

FIG. 15

FIG. 16 B

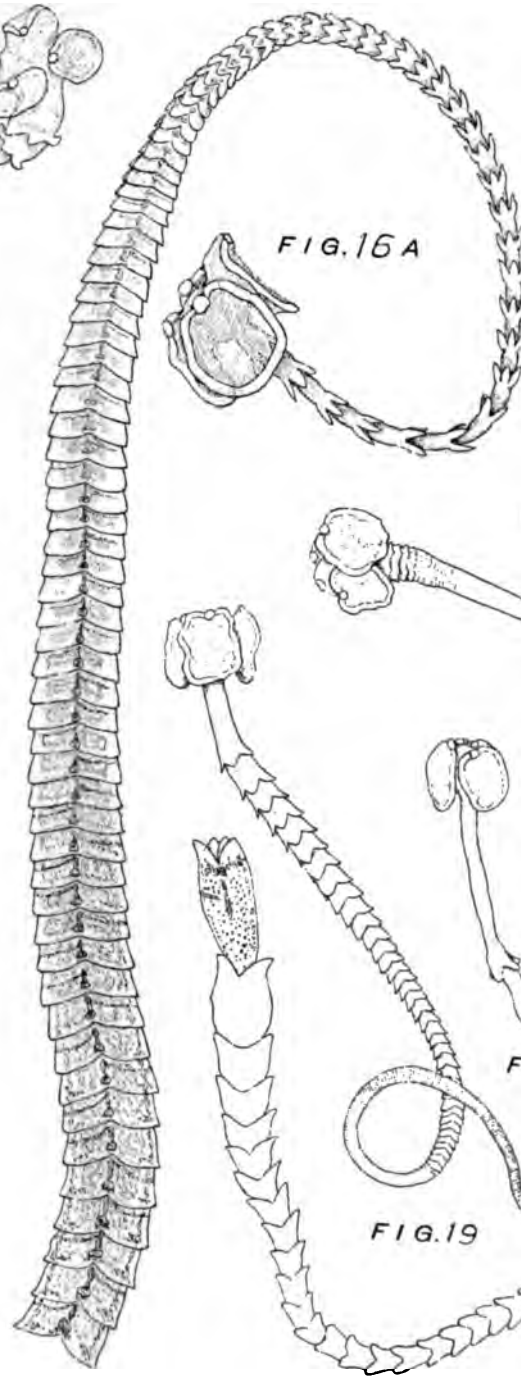

FIG. 16 A

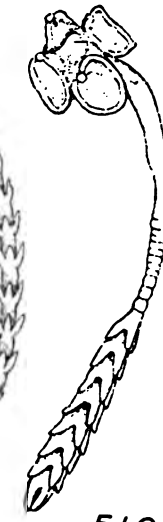

FIG. 17

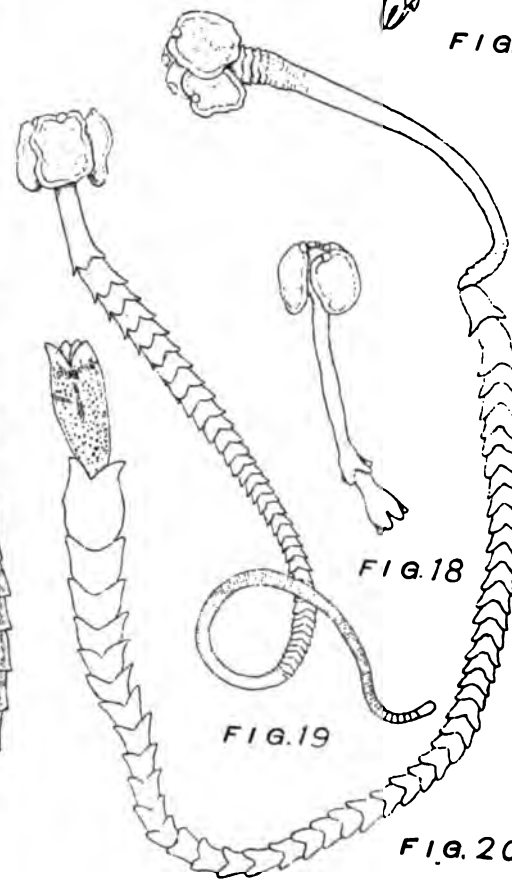

FIG. 18

FIG. 19

FIG. 20

PLATE VII.

Figures showing miscellaneous points in the proglottid formation of *C. laciniatum*. All from camera lucida drawings. The magnification is given for the figures as reproduced.

FIG. 21. A specimen which has just entered the "long-necked" condition. There are 34 old proglottids. Reproductive organs probably occur in front of the seventeenth proglottid, but as the specimen was stained for another purpose this cannot be determined with certainty. × 12 diameters.

FIG. 22. A "long-necked" specimen with a single one of the old "anterior" proglottids attached and segmentation beginning in the posterior part of neck region. × 12 diameters.

FIG. 23. Part of a young specimen showing a short non-segmented zone with an abrupt transition of the "anterior" proglottids into the zone. There were in this instance 30 "anterior" and upwards of 217 "posterior" proglottids. × 50 diameters.

FIG. 24. A "long-necked" specimen with a single "anterior" proglottid of the earlier chain still attached at the posterior end and the new segmentation well begun at either end of the neck region. × 12 diameters.

FIG. 25, *a*, *b* and *c*. To show the characteristic shapes of the "anterior" (*a*) and the "posterior" proglottids (*c*). Also the unsegmented zone which separates them. All from the same specimen. There were 35 anterior and 250 + posterior segments. × 50 diameters.

FIG. 26. Part of a transverse section through the non-segmented zone of a specimen about the stage of Fig. 7, Pl. I. See discussion on page 216 of the text.

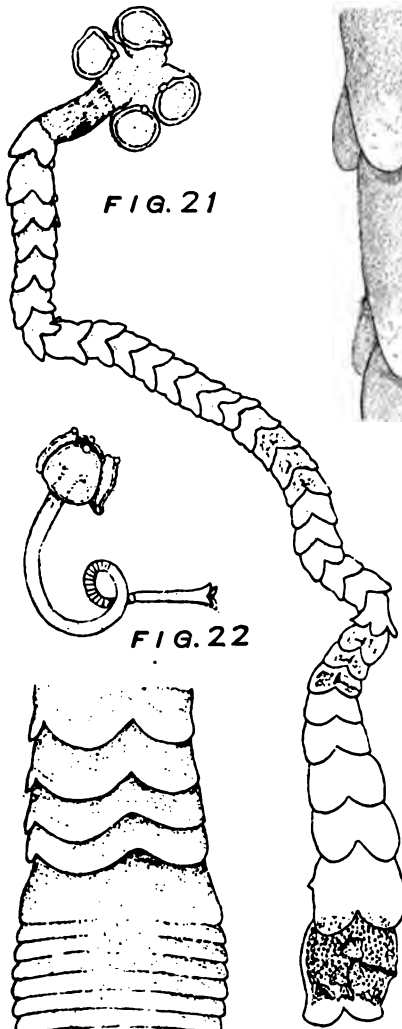

FIG. 21

FIG. 22

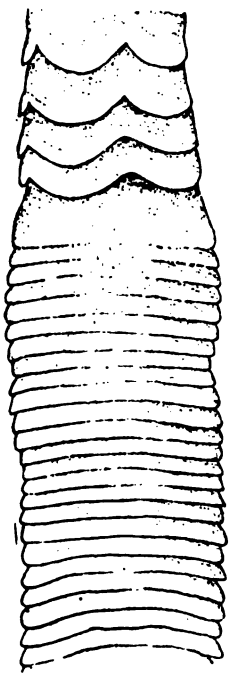

FIG. 23

FIG. 25 A

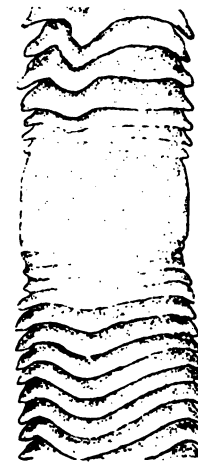

FIG. 25 B

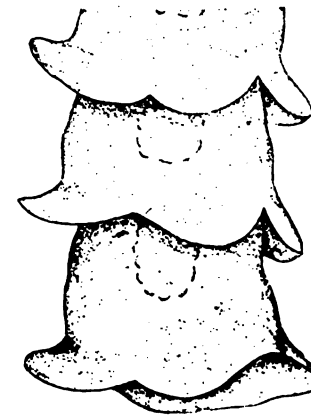

FIG. 25 C

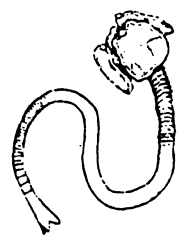

FIG. 24

FIG. 26

BIOLOGICAL BULLETIN

OF THE

Marine Biological Laboratory

WOODS HOLL, MASS.

VOL. XI OCTOBER, 1906 NO. 5

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BIOLOGICAL BULLETIN

THE PROTOZOAN LIFE CYCLE.[1]

GARY N. CALKINS.

Twenty years ago it was an amusing pastime to see with the aid of the microscope, and to describe, new and interesting forms of unicellular organisms. Today there is not a field of biological science that is not illumined by the deeper study of the protozoa, and the pastime of our fathers has become the science of protozoology to-day. In its modern aspect this new science has many sides, morphology, physiology, psychology, cytology, and pathology, and although there is little danger of its being cut up into unrecognizable parts, there is need of some ground principle or principles to hold the many branches of protozoa study together and to unify the science. It was the genius of Schaudinn, whose untimely death has taken from protozoölogy its most brilliant light, to establish such an unifying foundation, and in his clear perception of the importance of the life cycle we have the key note of our present day conception of the protozoa. With our present knowledge we may define protozoa as: Independent, unicellular, animal organisms which reproduce by division or spore formation, the progeny passing through various phases of activity collectively known as the life cycle, and manifesting various degrees of vitality with accompanying form changes.

In thus emphasizing the life cycle in the definition I would seek to introduce into protozoa study the recognition of the entire cycle of changes as a necessary basis for species. I would have the presentation of the life history of a protozoön a prerequisite for its acceptance as a new species and would have zoölogists recog-

[1] Substance of addresses given before Sections D and K of the British Association for the Advancement of Science, at York, 1906.

nize that a new species can no more be created on the basis of a single cell or group of cells in the same stage of the life cycle than a species of mammal can be established on the basis of a fore limb, a jaw bone, or a tooth. It seems to me high time that the science of protozoa-study should be freed from the charge of dilettantism, and time for the literature to be cleared of the great burden of synonyms that must ever increase so long as novices in this field of study see and describe in print what to them are new and curious forms.

There is enough known at the present time of protozoan life cycles to indicate that for a given species the cycle under similar conditions is always the same, and we are justified in considering the entire congeries of forms which the protozoön passes through in its life cycle, and not the single cell, as the "individual," comparable indeed, as has long since been pointed out, to the metazoön. There is nothing fanciful in comparing the rapid asexual phase of a protozoön with the proliferation of somatic cells of a metazoön, or the periods of conjugation and old age in a life cycle with the sexual maturity and senescence in metazoa. So many different forms are assumed by the protozoön in the numerous stages of vitality, that, unless the entire cycle is known, even the skilled observer might be justified in considering the various phases of the same organism as different organisms. Instances of this confusion come to your minds at once, and I need but mention Plasmodium and Polymitus, Coccidium and Eimeria to illustrate my meaning. The same confusion has recently come under my observation in connection with *Paramecium aurelia* and *P. caudatum*, which, since the classical work of Maupas, have been generally but erroneously accepted as distinct species.

The specific differences between these two supposedly different forms have been emphasized by Maupas and Hertwig, and more recently by Simpson, and are based upon some minor characteristics of size and form but mainly upon the presence of two micronuclei in *P. aurelia* and one in *P. caudatum*. In all cultures of *P. caudatum*, epidemics of conjugation appear at more or less regular intervals. Four pairs of conjugating forms were isolated from such a culture on March 11, 1905, and after separation the eight individuals were isolated and their several histories followed

out until the race in each case became extinct. Two of the eight individuals representing different pairs continued to multiply in cultures for more than a year, the method employed being the same as that used in previous experiments.[1] The other six died before reaching the fourth generation. One of the two successful exconjugants reorganized as *Paramecium aurelia*, the others as *P. caudatum*. The *caudatum* form was much more vigorous than the *aurelia* form and kept up in its division rate with a ninth individual that was chosen for control from the individuals that had not conjugated in the original culture. At the end of two months the *aurelia* form had divided 55 times, the *caudatum* form 76 and the control 77 times. In the period between the last of April and the end of June the *aurelia* form gradually lost its specific *aurelia* characters and became more and more like *caudatum*. The two micronuclei were reduced to one, one being absorbed, and by the seventieth generation the original *aurelia* form could not be distinguished morphologically from the original *caudatum* form, while its division energy became much greater than that of the other types in culture, an energy which lasted through more than 300 generations.

Without entering into a discussion of the interesting biological features of this apparent change of species which I have published elsewhere,[2] I would merely call attention to the fact that here at any time, during the first 45 generations at least, the thousands of cells that might have been formed would have been classified as *P. aurelia*, while study of the life history shows that it is only a temporary form assumed by *P. caudatum* and is to be interpreted as a mere variant or sport. These results merely emphasize the importance of studying the entire life cycle as a basis for protozoan species.

It is particularly important that these specific distinctions should be clearly recognized in that field of protozoan research which is most important at the present time, — the pathogenic. Here more than in any other field of biological study is the need

[1] Calkins, "Studies on the Life History of Protozoa," I., *Arch. f. Entwk.*, XV., No. 1, 1902.

[2] *Paramecium aurelia and Paramecium caudatum*, in "Biological Studies" by the pupils of William Thompson Sedgwick, Chicago, 1906.

of a clear conception of the different phases of the parasite, and the possibility of different hosts or of different effects on the same hosts at different periods of vitality should be known to the pathologists. The tendency to put protozoa on the same basis of research as the bacteria, despite the brilliant work in cultivating certain types of protozoa on artificial media, which one of my countrymen regards as the *sine qua non* of pathogenic protozoan research, seems to me to be a step in the wrong direction. To study parasitic protozoa in culture is to study them under one condition only, and in one phase only of the life history, and the different forms that are met with in such artificial media may be more often involution types than normal phases, and the great multiplication of species of *Trypanosoma* or *Spirochæta* bespeaks perhaps more than any other one thing the presence of a new type of novitiate in protozoan research.

If the life cycle is to be accepted as the basis of new species, and regarded as the individual in a taxonomic sense, it should be sufficiently definite to be taken as a unit, and the life histories of widely separated species should have some common grounds for comparison. Thanks to the great stimulus given in recent years to protozoan study, we know the full life history of many widely separated forms, and at the present time we are able to generalize to some extent and to formulate a few ground principles. The old-time comparison of the metazoön with the mass of cells that are formed by the repeated division of the first parent cell of a protozoön after conjugation, can be amplified and extended at the present time to the comparison with the metazoön of not only the mass or morphology, but the physiology or general biology of the constituent cells as well. As with the metazoön so with the aggregate of protozoa cells, we note a period of youth characterized by active cell-proliferation; this in both groups of organisms is followed by the gradual loss of the division energy accompanied by morphological changes in type of the cells preliminary to conjugation and fertilization and to the renewal of vitality by this means. When such renewal is omitted, and for one reason or another this stage is never reached by the great majority of protozoa, the third characteristic period — old age — supervenes and the race of pro-

tozoa dies out from protoplasmic senility no less surely than does the body of the metazoön. We can distinguish, then, in the life history of a protozoön three more or less clearly marked stages, youth, adolescence, and old age, each with certain characteristics but which we cannot sharply mark off one from another any more than we can clearly limit the three stages of a metazoön.

As with the fertilized egg of a metazoön, the copula or fertilized cell of a protozoön is endowed with a great power of cell-reproduction and with a high potential of vitality, and this is the main characteristic of the first period of the life cycle. This reproduction may take the form of simple division, of budding, or of spore formation, according to the difficulties that have been successfully overcome by the species in its struggle for existence. The young forms show well marked conformity to type, and this feature, occurring when the greatest numbers of representatives of the species are in evidence, undoubtedly has given a false impression of the stability of form of the protozoan species.

This is the period also of the greatest resistance to adverse conditions of the surrounding medium and in pathogenic forms it is the period of greatest malignancy. It is a well-known fact that, in many parasitic forms of protozoa, attempts to inoculate from animal to animal are either failures altogether or result in a weakened race of the organisms; these failures are perhaps due to the inability of the organisms in a more or less weakened condition to withstand the natural immunity of the host, which they are perfectly able to do with the full potential of vitality with which they are endowed after conjugation. In some cases, as for example in Trypanosoma, the natural vitality of the parasite is so much greater than the natural resistance of the host that such inoculation is possible and the transplanted organisms continue to live. The matter of malignancy is so intimately connected with this question of restored vitality that, in yellow fever for example, it alone is almost sufficient to indicate that conjugation processes must take place in the body of *Stegomyia fasciata.*

This first period is then marked by a distinct excess of constructive over destructive metabolism and in the series of divisions or repeated spore formation which follows fertilization there is a

gradual tendency for the energy of multiplication to weaken, or, as we may express it, the constructive and destructive processes tend to equalize. With the decline of the division rate comes the advent of the second period in which the most important functions in the life of the protozoön occur.

The main characteristic of this second general period, or adolescence, is a general decline in the multiplication rate and more or less definite change in form of the cells and in their chemical and physical composition. A single cell, unless it is in the sexual phase, gives little or no clue to its stage in the life history. In the majority of cases it is only by the study of a long series that the student is able to recognize this period in the life cycle. In such a study, which one can easily carry on with certain free forms of ciliates, the decline of the division rate with advancing age of the series is easily followed. In a form like Paramecium, for example, where there is no sexual dimorphism, one notes at this period a change in the physical constitution of the protoplasm. Such changes, leading in Paramecium to what I have termed the "miscible" state, are certainly the most striking features in the life history of protozoa and *a fortiori* of the period of adolescence, for in them we find an explanation of all the form changes that take place in any life history. Such form changes may involve only the cytoplasm as in *Paramecium* or *Tetramitus*, only the nucleus as in *chromidium* formation in rhizopods, or both nucleus and cytoplasm as in gamete formation with or without sexual differentiation.

In *Paramecium* this condition comes at a period which Maupas designated as sexual maturity. The body size is somewhat less than at earlier periods, although this is by no means a constant feature, size depending chiefly on the rate of division and so only indirectly on age. The plasticity at this period is remarkable, and the cortical plasm is so sticky that two organisms striking each other will fuse at the point of contact. I have had *Paramecia* in culture during an epidemic of conjugation with the protoplasm so highly miscible that amorphous groups of partially fused *Paramecia* were formed by the accidental union of from three to nine individuals, dozens of such groups whirling round and round amongst the normally conjugating pairs.

In *Tetramitus*, *Cercomonas*, or other similar flagellates with a firm contour and a definite shape, the organisms in the period of adolescence become plastic or even amœboid, and like *Paramecium* they conjugate while in this condition.

Among the morphological changes that occur during this period of adolescence none are more subtle or more difficult to interpret than those of the nucleus; indeed, we are here brought face to face with one of the fundamental problems of modern biology — the maturation phenomena. While these are undoubtedly general biology or cell problems, there is another phenomenon connected with the nucleus of protozoa at this period which, despite the ingenious analogies of Goldschmidt, has no satisfactory simulacrum in metazoa, — the formation of *chromidia*. Using the term *idiochromidium* proposed by Mesnil to designate the distributed chromatin prior to gamete formation, we see in this material a practically characteristic sexual substance which, appearing prior to conjugation, belongs primarily to this period of adolescence. In all those forms in which multiple fragmentation has been described, the fragments become the nuclei of conjugating gametes (examples in *Coccidium schubergi* and *Entamœba* according to Schaudinn). In rhizopods, however, especially in the testacea, this nuclear distribution begins at an earlier period in the life history, and the *idiochromidium* is characteristic of the ordinary vegetative forms (examples in *Difflugia* [Zueltzer], *Centropyxis* [Schaudinn], etc.). This chromatin differentiation and distribution indicates a curious change in the chemical balance of nucleus and cytoplasm during the period of adolescence; a change which may appear earlier or later in different types, the earliest appearance being seen in the case of infusoria where the differentiation into vegetative and germinal nuclei occurs immediately after conjugation. In the majority of cases, however, the appearance of the *idiochromidium* and the various stages leading up to its formation may be taken as evidence of advancing age of the series of individuals and as a token of the near completion of the cycle.

So widespread is this nuclear phenomenon and so well established in protozoa studies that we are justified in some cases in arguing from this phenomenon alone that adolescence and the

period of sexual union is imminent. For example, in the case of the ordinary forms of *Amœba* it is remarkable that sexual processes have never been observed, although the engulfing of one individual by another has been interpreted as a conjugation phenomenon. One series of forms has recently come to my notice which seems to indicate a sexual process. The ordinary form of *A. proteus*, usually has but a single nucleus, but in one culture in my laboratory after a long series of uninucleated forms a culture appeared in which the individuals were in various phases of encystment or in nuclear fragmentation immediately prior to encystment. It was found that the single primary nucleus divides by mitosis; that these divide again and so on until as many as seventy large nuclei may fill the body of the amœba. In some cases before this number is reached the larger nuclei begin to break down into large granules which become distributed throughout the cell appearing exactly like the idiochromidium of *Difflugia*, *Arcella* or *Centropyxis*. All but one of the primary nuclei are finally disposed of in this way, that one remaining unused even in the final encysted stage. In this final stage the cyst is filled with many reproductive bodies, which from analogy with other rhizopods I interpreted as gametes.

Not only is chromidium formation important in determining the phase of development of a given form, but it may also be of the greatest assistance in proving the protozoön nature of questionable structures found in certain diseased tissues. An interesting example has recently come up in connection with the organism of rabies. This organism, under the name of the Negri bodies, has been looked upon with great suspicion by biologists and pathologists alike, and from its general staining reaction and from its ordinary vesiculated appearance it has been more frequently passed by as an artifact or secretion or degeneration product, than considered as an organism. But during the season just passed, Dr. A. W. Williams, of New York, working with a different method from that ordinarily employed, was able to prove that what appear usually as vesicles in these bodies are in reality substances which take a characteristic nucleus stain with the Giemsa method, and she shows that the Negri bodies are amœboid cells with nuclei in different stages of chromidium

formation. These erstwhile questionable bodies must now go into the protozoan literature under the generic name of *Neuroryctes* given by Dr. Williams. When the same method comes to be applied to the small-pox organism, I am confident that the last doubter will be convinced that it, too, is a protozoön, and of the rhizopod type.

Still another feature of this second period is the change in form of the conjugating individuals. We have seen that, with increasing maturity, the organisms of a cycle lose their definite shape, become plastic or amœboid in some cases and conjugate while in this condition. Now it is probably due to the same underlying causes that gametes of relatively minute size are formed. In *Polytoma*, for example, size differences are entirely facultative, two normals, two reduced individuals, or one normal and one reduced individual may unite. From such an indifferent condition we find all intermediate stages to fully established obligatory differences in size between conjugating forms and vegetative forms. This might be interpreted as a purely physiological matter depending upon the general chemical and physical balance in the cell, but with it is bound up very often a second phenomenon,—sexual differentiation, which has a deeper significance than the mere change of form at this period of adolescence. The study of protozoa has thrown no light as yet on this problem, which according to recent experimental and cytological findings, especially Wilson's discoveries on the extra sex-determining chromosome in certain insects, would seem to be a matter of inheritance rather than of controllable physiological balance. In many cases what may be called secondary sexual characters in protozoa are evident from the very outset after conjugation, even the first progeny being sexually differentiated as in *Trypanosoma* or *Adelea*, and this certainly can not be traced to advancing age or to changes in chemical relations of nucleus and cytoplasm.

In some cases these differentiations are not established until some change in external conditions brings them out. Klebs, Dangeard, and others have made different types of flagellates conjugate by changing the temperature or increasing the density in the surrounding medium. The same experiment is performed by mosquitoes and other insects on various parasitic

protozoa, as when some blood dwelling parasite is withdrawn from the hot environment of the mammalian body to the colder regions of a mosquito's digestive tract. If such experiments become obligatory, and it is apparently so in many cases, then certainly the most efficient prophylaxis is getting rid of the all-important intermediate host, a preventive measure that has been so signally successful in the Roman Champagna, in Cuba, in Vera Cruz, and in New Orleans.

The happenings within the body of such intermediate hosts are by far the most important to the parasitic protozoa of all their life processes, for the conjugation period with them, as with all free-living protozoa, is the critical period of the life history, and on it depends whether the race shall be given a new vigor and a new lease of life, or shall pass on into the third period of the life cycle characterized by old age or senescence and death.

The third period in the life cycle of protozoa is characterized by the peculiar cytolytic processes that accompany starvation, by loss in size, by vacuolar degeneration in nucleus and cytoplasm, and by final natural death. The symptoms may precede both physiological and germinal death, and many of the so-called involution forms frequently described in parasitic protozoa may be individuals in this third stage of vitality. At this period, conjugation, as pointed out by Maupas, seems to be impossible, the chance of rejuvenescence is cut off, and the race, now comparable to the worn out somatic cells of a metazoön, becomes extinct.

This series of changes from the fertilized cell to the ultimate extinction by natural death is a consecutive series and forms a clean-cut and well-defined life cycle or unit for all forms of protozoa. The vital processes are vegetative in nature and varying phases may be largely accounted for by the conditions of metabolism. In metazoa we can make a clear distinction between the history of the individual and the history of the race, and in protozoa, with the life cycle as the unit, we can make the same distinction. The ordinary phenomena of vegetative life of the cell, metabolism in all its processes, have to do with digestion, excretion, irritability, growth, and multiplication, and are functions pertaining distinctly to the life cycle and may be considered independently of those which have to do with the continuity of the

species or race. Among the latter are the many processes accompanying fertilization and rejuvenescence, and the phenomena here, although not a part of the life cycle any more than sexual processes are a part of the life history of an individual metazoön, are nevertheless dependent on it and can not be omitted in any adequate account of a life cycle, since the succession of cycles, or the race, depends upon them. It is in this field of phenomena that we find the most fascinating aspects of modern protozoa study, for the problems here are general biological problems, and their solution means the illumination of some of the darkest places in biological science. Let me ask your attention for a few minutes, in concluding, to some features of this general subject that have interested me during the past year. They relate to maturation phenomena, to renewal of vitality after conjugation, and to artificial rejuvenescence in *Paramecium*, all functions of the species or race rather than of the unit life cycle.

One of the deepest problems of general biology, heredity, is bound up with the history of the chromatin in the formation of germ cells, and here in protozoa, as the culminating phenomena of the period of adolescence, we find the same type of maturation as in metazoa or the higher plants. At the present time there seems to be no connection whatsoever between the phenomenon of chromidium formation and maturation, while the residual masses of chromatin that are left to degenerate and disappear in so many protozoa prior to fertilization, are more nearly comparable with the residual chromatin of a germinal vesicle in a metazoön than with maturation of the chromosomes. When the history of the idiochromidium is more perfectly established, we may have further evidence to support the identity of the processes in protozoa and metazoa. The division of the chromidium granules in *Amœba proteus* [1] which I have elsewhere described in some detail may be the equivalent of the maturation divisions of the chromosomes in germ cells of the metazoa. There is more definite evidence of this similarity in other forms of protozoa. In *Trypanosoma noctuæ*, for example, maturation processes entirely similar to those of the higher animals have been de-

[1] "Evidence of a Sexual Phase in the Life Cycle of *Amœba proteus*," *Arch. f. Protist.*, Bd. V., 1904.

scribed by Schaudinn. In his preliminary paper this brilliant observer does not go into the details of the process but states categorically that the male nucleus contains only four of the typical eight somatic chromosomes, while the female nucleus contains a similar four in the shape of tetrads formed by the transverse division into four parts of a longitudinally split spireme thread, which are reduced to four single chromosomes by two successive divisions, one a reducing the other an equation division. Cytologists everywhere are waiting for a confirmation and for a more definite description of this remarkable process which thus resembles very closely the maturation processes of certain metazoa. Other instances of reduction in protozoa have been given from time to time, and are quite sufficient to show that maturation phenomena are as widespread in protozoa as in other forms of life and that their underlying significance is as wide as the entire field of biology. The infusoria perhaps more than other forms of protozoa are generally cited in this connection. Here Maupas early showed that of the divisions of the micronucleus of *Paramecium caudatum* one persists to form the functional male and female pronuclei, while the other three atrophy and disappear in the cytoplasm. Schaudinn showed that in Heliozoa one daughter-nucleus which he compared with a polar body is thrown off to disintegrate and disappear in conjugating Actinophrys, while Hertwig showed that two such bodies are cast off by conjugating individuals of Actinosphærium.

In none of these cases has the finer details of chromosome formation been sufficiently described, and the number of chromosomes has rarely been counted or the actual reduction made out. Hertwig somewhat doubtfully claimed that the number in *Paramecium* is reduced from eight or nine to four or six, but there certainly must have been a mistake in the interpretation of what constitutes the chromosome in this case, for the actual number is many times greater than what he gives. One of the graduate students at Columbia, Miss Cull, has worked with me the past year on the formation of the chromosomes during the conjugation period of *P. caudatum*, and although our results on the maturation divisions are not yet ready for publication, we have proved that the history of the chromatin in the early period

of the maturation process agrees with a remarkable exactness with what occurs in many germ cells. The curious and enigmatic crescent which the micronucleus forms during the early phases of maturation, is the form assumed by the nucleus during the stages of synapsis and contraction, and the first spindle develops from this crescentic nucleus with its longitudinally divided chromosomes in the form of heterotypical loops. The condensed chromatin of the resting nucleus is first broken up into many fine granules which become arranged in lines radiating backwards from the intranuclear division center. The nucleus elongates in the direction of these lines until it is seven or eight times the original length. Then, with the growth of the division center, the entire structure becomes crescent shaped and many times the volume of the original micronucleus. The long lines of chromatin appear to form a confused network, but in the contraction phase which follows shortly after the crescent, it can be seen that these lines of chromatin are much thicker than they were and distinctly double, and although not conclusively demonstrated, the most reasonable interpretation regarding their origin is that the long lines of chromatin unite side by side in a typical parasynapsis.

It has been customary to describe the pronuclei in *Paramecium* as fusing while in the spindle form. In a general way this is true, but it is only an elongated form assumed by the nucleus at the time of this union, for the spindle at this time is in no sense a mitotic spindle, the chromatin being in a finely divided state and distributed throughout the nuclei.

It has been generally believed that conjugation brings about a renewal of vitality, a Verjüngung or rejeunissement according to Bütschli and Maupas, or an Erfrischung, to use a term suggested by Weismann. This interpretation seems to be so obvious on *a priori* grounds that experiments to prove it would appear hardly necessary. In protozoa it is not rajuvenescence strictly speaking but the formation of a new individual, and so also is it in metazoa. It would seem to be easy enough to prove that conjugation actually starts a new race from weakened individuals, but singularly few experiments have been undertaken with this object in view. Some that have been carried out by Miss Cull

and myself during the last year, while proving that conjugation does bring about renewed vigor, also show that the interpretation must be trimmed of some of its generalizations.

It has also been generally assumed in cases of conjugation where, as in *Paramecium*, both individuals are similar in size, and where conjugation is only temporary, that both individuals are fertilized, but according to these experiments which I have cited Miss Cull has shown that, in the majority of cases, while one individual of the original pair is markedly vigorous after conjugation, the other one either forms a weak strain or dies off at an early period.

It would seem from these results that in cases like this of isogamous conjugation we can catch a glimpse at least of the same principle that operates in the fertilization of an egg by a spermatozoön, where one cell loses its identity and continues to exist only in conjunction with another cell. It appears to be a case of incipient fertilization and indicates some physiological difference between the conjugating individuals analogous to that between spermatozoön and egg.

The analysis of the conditions governing conjugation has not yet been carried very far. My own experiments show that Maupas's three conditions can not hold. Hunger apparently has nothing to do with it, and diverse ancestry is not essential, for I have obtained as large a percentage of successful endogamous as exogamous pairings and have carried one endogamous exconjugant through 379 generations. Maturity, however, the third "condition" postulated by Maupas, seems to be necessary, understanding by this term the peculiar state of cytoplasm and nucleus when conjugation is possible and a condition which can be induced by artificial means such as change in temperature or of density in the surrounding medium.

There is yet another matter that I wish to speak of in connection with conjugation and rejuvenescence, and that is the question of artificial rejuvenescence, a matter which has an important bearing, it seems to me, in all protozoan life histories. In a series of experiments which I carried on for twenty-three months with one race of *Paramecium*, it was found that periodic reductions of vitality occurred at intervals of about six months. At such

periods of "depression" the race under cultivation would have died out entirely, had not stimuli in the form of extracts of different substances (beef, pancreas, brain, etc.) been applied. With the aid of such restoratives on three different occasions, the race was carried through four "cycles" of activity and through 742 generations.

There is no doubt that the organisms would have died of a real physiological exhaustion, had they not been artificially stimulated, and even when the race finally ran out, it was not from physiological exhaustion in the same sense, for stimuli had again been successful in restoring the vegetative functions. It was due to some more deeply lying trouble, and the results confirmed Hertwig's view of "germinal" death as contrasted with "physiological" death, and, as I have elsewhere pointed out, they demonstrate that in the protozoön as in the metazoön we may distinguish between somatic and germinal protoplasm.

We can readily understand how such periods of depression may be overcome in nature and stimulation effected by changes in the immediate environment or, in parasitic forms, by changes in the blood, and continued activity of certain parasites or appearance of the recidive in malaria, etc., may thus be accounted for. But the organisms themselves have an efficient means of bringing about this renewal of vitality. It seems probable that some original supply of physiological energy is continually drawn upon by the vegetative organisms, some "potential of vitality," which, like the charge of a battery, may become exhausted. As in a battery this potential can be renewed by artificial means, but unlike a battery it can also charge itself by the process of parthenogenesis. This has been shown by Schaudinn to take place in the malaria organism, *Plasmodium vivax* and in *Trypanosoma noctuæ* of the owl. Here, as in some insects, rejuvenescence is brought about by the union of the kinetonucleus and the vegetative nucleus, and is quite analogous to the fertilization in some metazoa of the egg nucleus by a polar body nucleus. Protozoan organisms which are thus restored by parthenogenesis seem to carry with them at least one extra charge of vitality, and we have no basis for speculating as to the length of time that such parthenogenetic processes may continue in a race. In the experi-

mental work on Paramecium artificial parthenogenesis was successful at three different periods of depression but failed on the fourth, and we may infer that normal parthenogenesis has only a limited success and that sooner or later the race must come to an end unless conjugation and nuclear reorganization take place. It is conceivable that parthenogenesis is only a means of offsetting physiological death or in other words, of stimulating physiological activities in protoplasm in which the potential of development is not yet exhausted.

LOCOMOTION IN YOUNG COLONIES OF PECTINATELLA MAGNIFICA.

ALICE W. WILCOX.

Pectinatella magnifica is the largest of the fresh-water bryozoa (Phylactolæmata). It is a distinctively American form and is generally known by the conspicuously large masses of it, which are formed in the late summer and fall. These masses commonly are as large as a man's head and often attain the size of sixteen by eight or nine inches. They are found floating or attached to submerged solid material in some of our purest fresh-water ponds and reservoirs. Each of these masses consists of a thin coating of *Pectinatella* colonies attached to a thick substratum of transparent, colorless jelly. The colonies are diamond-shaped in general form, with many slender, radiating lobes, each bordered by a double row of actively-contracting polypides.

These jelly masses are one of the most unique phases of the life-history of the species. In order to understand their mode of formation, I undertook a preliminary study of the growth and behavior of the young *Pectinatella* colonies. This resulted in the discovery that during their early stages these colonies have the power of independent motion. This paper gives the evidence of this fact of *locomotion in young Pectinatella colonies.*

It was long believed that the power of locomotion in the bryozoa was confined to the genus *Cristatella*, of which it is a striking characteristic. But a Danish zoölogist, Wesenburg-Lund ('96), discovered that another genus of the Phylactolæmata, *Lophopus*, has this power of independent locomotion. He observed that the young *Lophopus* colonies sometimes migrate six centimeters in the course of twelve hours.

In 1901 I studied young colonies of *Pectinatella magnifica* under most favorable conditions at Cold Spring Harbor, L. I. There *Pectinatella* occurs in abundance in three connecting fresh-water ponds. Flood-gates separate the adjacent ponds and to these the statoblasts of *Pectinatella* often attach themselves and

give rise to young colonies. The colonies on one gate I observed regularly at twenty-four hour intervals for a period of six weeks from June 8 to July 21. The observations were recorded in a series of forty-three outline drawings made to scale, showing the colonies in their actual position in relation to certain fixed lines. A selected series of fifteen of these figures is reproduced to illustrate this paper.

In each figure, the unbroken outlines represent the position of the colonies on the date indicated, and the broken outlines their position the previous day. The two straight lines, crossing each other at right angles represent the fixed reference lines on the flood-gate.

Fig. 1 represents two small colonies, *A* and *B*. Colony *A* has just come out of its statoblast and become attached. Colony *B* is somewhat older and about three times as large as *A*.

Fig. 2 represents the same colonies two days later. Their size is not perceptibly increased, but both colonies have moved to the right and *A* has approached a little nearer to *B*. *B* shows a slight constriction preliminary to division.

Fig. 3 represents colonies, *A* and *B*, after another interval of three days. Both colonies have increased in size and are coming still nearer together. *B* is becoming definitely lobed.

Fig. 4 represents the conditions one day later. Colony *A* has migrated back toward its original position. Colony *B* has divided by fission into two parts, B^1 and B^2.

Fig. 5 represents the colonies the following day. Colony *A* is still shifting its position slightly, but without increasing in size. Colonies, B^1 and B^2, are already beginning to move apart and to change their position in relation to colony *A*.

Fig. 6 represents the conditions seven days later. After a period of comparative rest colony *A* has moved to a new position and increased in size. Colonies, B^1 and B^2, are still changing their absolute and relative positions.

Fig. 7 represents the colonies three days later. Each has definitely enlarged and changed its position somewhat. B^2 has moved entirely off its position of the day before as shown by comparing the even and broken outlines of the colony.

Fig. 8 represents the conditions after another interval of three

days. It shows further increase in size and a greater degree of locomotion of each of the colonies.

Fig. 9 represents the colonies three days after Fig. 8. Marked changes have occurred; colony A has more than doubled its size and has divided into two parts: A^1 and A^2, each of which is moving; A^1 is growing and migrating; B^2 has just divided into two parts: $B^{2.1}$ and $B^{2.2}$.

Fig. 10 represents the colonies two days after Fig. 9. They are in a state of rapid growth as indicated by their increased size and their definitely-lobed margins.

Fig. 11 represents the colonies two days later, indicating important changes. Colony A^1 is divided into two: $A^{1.1}$ and $A^{1.2}$, which are moving apart. Colony A^2 is much enlarged and divided into $A^{2.1}$ and $A^{2.2}$. Colony B^1 has become deeply lobed. Colony $B^{2.1}$ is moving and preparing to divide. Colony $B^{2.2}$ has divided into colonies $B^{2.2.1}$ and $B^{2.2.2}$.

Fig. 12 represents the conditions three days later. It shows increase in size of each of the colonies, and further division of colonies $A^{2.1}$, $A^{2.2}$, B^1 and $B^{2.1}$. Also a part of colony $B^{2.2}$ has fused with a part of colony $A^{2.2}$. From this time forward the locomotion of the individual colonies cannot keep pace with their growth. Hence as the colonies divide and move apart they come in contact with other colonies with which they fuse.

Fig. 13 represents the colonies two days after Fig. 12. A number of the colonies are literally running together.

Fig. 14 represents the conditions after an interval of three days. It shows marked growth in every direction. All but a few outlying colonies are united in a single mass.

Fig. 15 represents the colonies three days later. They are forming one continuous mass, which is not only expanding its area rapidly, but also is thickening perceptibly, especially in the middle. The latter change is due to the heaping up of the secretion of the under surface of the colonies.

This study of the young *Pectinatella* colonies shows very conclusively that they possess the power of *locomotion*. This power is definitely associated with the phenomena of growth and division. The increasing size of a colony causes it to divide. The fact that young *Pectinatella* colonies multiply by fission was first

brought out by Hyatt (65). The frequent division of the colonies along with the tendency toward further growth occasions locomotion.

The real cause of locomotion must however be referred back to the activity of the moving colonies and the condition of the substratum on which they rest. The individual polypides of *Pectinatella* are very irritable. They contract and expand frequently and with some force. All the polypides on one side of a colony contracting simultaneously give impetus enough to move the whole colony over a slippery surface. The gelatinous secretion which underlies the young colonies has not yet hardened, and in its semi-fluid state offers a slimy surface over which the small colonies move with very little resistance.

The rate and amount of locomotion varies with the size and condition of the colony. After a colony is well started and growing rapidly, it divides often and the resulting colonies move apart quickly and as far as the free space about them permits. But there are limits to this power of locomotion. The colonies can move only as they are impelled by the activity of the polypides. Hence as the polypides lose their vigor, as they do during reproduction, locomotion must cease. The power of locomotion also decreases with the increasing size of the colonies. The external conditions also limit locomotion. So long as the gelatinous substratum is in a semi-liquid state, the young colonies move with freedom, but as it gradually solidifies they become fixed. Often locomotion is limited also by the space about the colony. If two colonies in process of locomotion come in contact with each other, their motion is checked and they fuse together. As the colonies spread and form a confluent mass, further growth is allowed for only by the rapid thickening of the gelatinous ectocyst.

But what is gained by this unique power of locomotion? As division is necessary to keep the individual colonies small, so locomotion after division, is necessary to allow growth to proceed freely in all directions, and the characteristic radiately-lobed colonies to be formed.

This discovery of locomotion is significant, owing to the light which it throws upon the mode of development of the large

fall masses of *Pectinatella magnifica* already referred to. The only theory of their origin, on record up to this time, is that of Hyatt ('65), who believed that each mass was derived from as many statoblasts as there are colonies. In the light of this study, it is evident that each mass arises from relatively few, instead of innumerable statoblasts, and that it is formed in a similar way to the mass resulting from the two colonies, *A* and *B*, that is, through the combined phenomena of growth, division and locomotion.

BROWN UNIVERSITY,
ANATOMICAL LABORATORY, June, 1906.

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Description of Plates.

Plate VIII.

Fig. 1 represents *Pectinatella magnifica*, colonies: *A*, *B*. July 8.
Fig. 2 represents *Pectinatella magnifica*, colonies: *A*, *B*. July 10.
Fig. 3 represents *Pectinatella magnifica*, colonies: *A*, *B*. July 13.
Fig. 4 represents *Pectinatella magnifica*, colonies: *A*, B^1, B^2. July 14.
Fig. 5 represents *Pectinatella magnifica*, colonies: *A*, B^1, B^2. July 15.
Fig. 6 represents *Pectinatella magnifica*, colonies: *A*, B^1, B^2. July 22.
Fig. 7 represents *Pectinatella magnifica*, colonies: *A*, B^1, B^2. July 25.
Fig. 8 represents *Pectinatella magnifica*, colonies: *A*, B^1, B^2. July 28.
Fig. 9 represents *Pectinatella magnifica*, colonies: A^1, A^2, B^1, $B^{2\cdot1}$, $B^{2\cdot2}$. August 1.

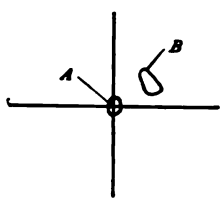

FIG. 1.

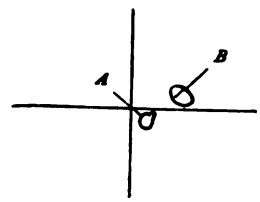

FIG. 2.

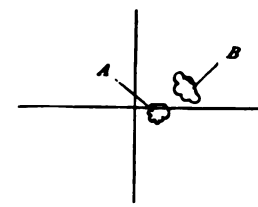

FIG. 3.

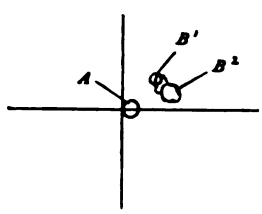

FIG. 4.

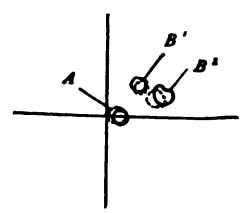

FIG. 5.

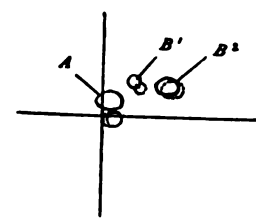

FIG. 6.

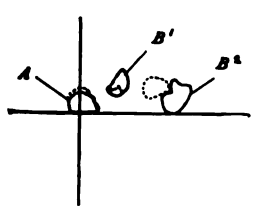

FIG. 7.

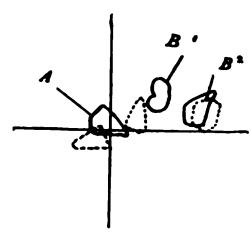

FIG. 8.

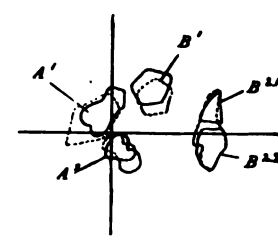

FIG. 9.

PLATE IX.

Fig. 10 represents *Pectinatella magnifica*, colonies: A^1, A^2, B^1, $B^{2\cdot1}$, $B^{2\cdot2}$, August 3.

Fig. 11 represents *Pectinatella magnifica*, colonies: $A^{1\cdot1}$, $A^{1\cdot2}$, $A^{2\cdot1}$, $A^{2\cdot2}$, B^1, $B^{2\cdot1}$, $B^{2\cdot2\cdot1}$, $B^{2\cdot2\cdot2}$. August 5.

Fig. 12 represents *Pectinatella magnifica*, colonies: $A^{1\cdot1}$, $A^{1\cdot2}$, $A^{2\cdot1\cdot1}$, $A^{2\cdot1\cdot2}$, $A^{2\cdot2\cdot1\cdot1}$, $A^{2\cdot2\cdot1\cdot2}$, $A^{2\cdot2\cdot2}$, $B^{1\cdot1}$, $B^{1\cdot2\cdot1}$, $B^{1\cdot2\cdot2}$, $B^{2\cdot1\cdot1}$, $B^{2\cdot1\cdot2}$, $B^{2\cdot2\cdot1}$, $B^{2\cdot2\cdot2}$. August 8.

Fig. 13 represents the same colonies as Fig. 12 fusing. August 10.

Fig. 14 represents the same colonies as Fig. 12 fusing. August 13.

Fig. 15 represents the same colonies as Fig. 12 fusing. August 16.

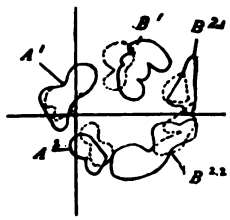

FIG. 10.

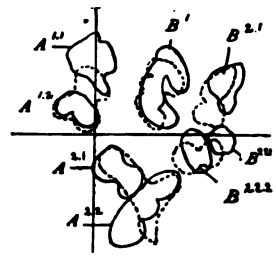

FIG. 11.

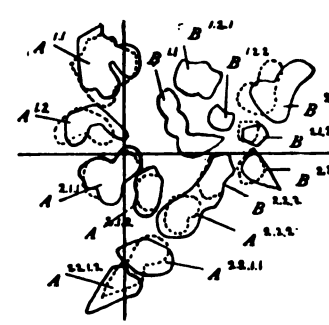

FIG. 12.

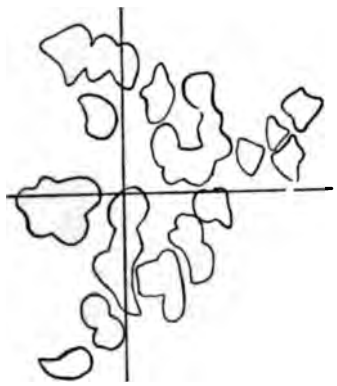

FIG. 13.

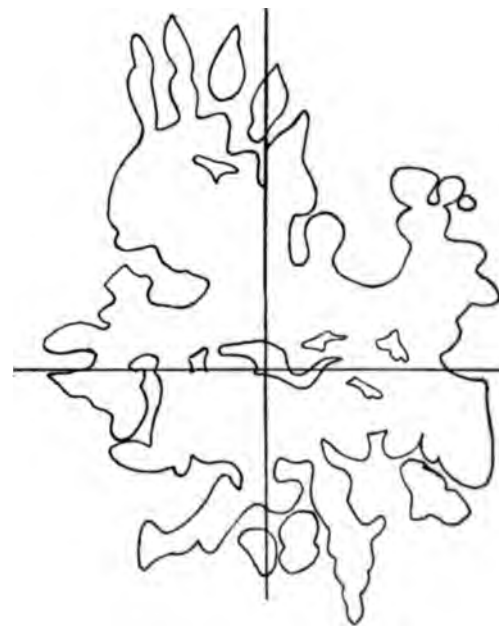

FIG. 15.

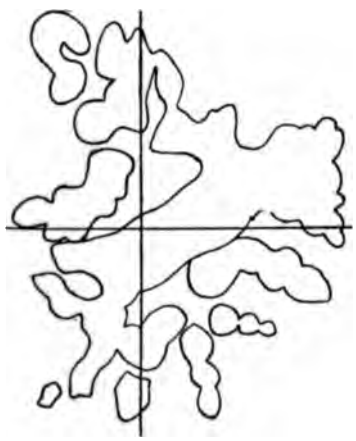

FIG. 14.

ON THE RELATIVE DIMENSIONS OF THE OSSEOUS SEMICIRCULAR CANALS OF BIRDS.[1]

MAY AGNES HOPKINS.

The purpose of this paper has been to determine whether there is any relation of the comparative dimensions of the bony semicircular canals of the ear of birds, either to mode of locomotion, or to genetic affinities.

These precise questions have apparently not been considered, except in a short paper by Farrar (1831) that was inaccessible to me. Indeed there has been no extended comparative study of the dimensions of these bony canals, though the inner ear labyrinth has been carefully investigated, notably by Retzius (1884).

In all cases my dissections were made on dried adult skulls. The material used was the collection of the University of Texas. All the American specimens studied had been identified by Prof. Montgomery, and exotic ones by the dealers from whom they were secured.

The work was done entirely under the direction of Prof. Montgomery, to whom I am greatly indebted for his valuable assistance and encouraging sympathy.

1. MODES OF LOCOMOTION IN BIRDS.

Archæopteryx, the earliest known bird, was a good flyer if we may judge from its possession of a sternal keel, but it also used its fore-limbs as grasping organs since they were provided with free unguiculate digits.

What has given birds their superiority over the reptiles was the acquisition of flight, and the main avian peculiarities are referable to this mode of locomotion. Birds may have developed flight in one of two ways — by saltatory locomotion, whereby the hind limbs were used as propellers, and the fore-limbs as organs of balance; or by scansorial locomotion on trees, whereby

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the fore-limbs came to be used as parachutes in the aerial passage from one tree to another. The second of these ways may have been the more probable. No modern birds use their wings in the manner of legs, except the young of *Opisthocomus*, but employ them rather as organs of flight, as balancers or as flippers (penguins).

The different modes of locomotion are intimately connected. In this study of the bearing of the relation of the size of the semicircular canals to the mode of locomotion, the following main kinds of locomotion were distinguished: The *cursorial*, where the hind limbs are employed to the greatest extent, and upon the hard ground; of such birds I have examined the ostrich and roadrunner. The *arboreal*, where wings and feet are employed about equally, as in most of the passeres. A modification of the preceding is the *scansorial* locomotion of such birds as woodpeckers. The *volant*, where the wings are used to the greatest extent, as in all birds of long sustained flight; those with the power of soaring represent the acme of this mode of locomotion. The combination of *volant* and *nektant*, that is of strong flight with the power of swimming, as in the gulls and albatrosses. The *pygopodal*, which may be swimming under water (grebes), or flight under the water (penguins). The *grallatorial*, where the birds walk upon moist or yielding ground, with an elongation or partial webbing of the toes; such birds have either a strong or weak power of flight and some of them can swim to a slight extent.

In the evolution of these modes of locomotion a climbing, arboreal habit was probably earliest. From this would have followed divergently: (*a*) development of stronger flight, and more aerial life; (*b*) loss of flight and terrestrial running; (*c*) development of a wading habit. Swimming birds have probably descended from waders and divers from swimmers. The most modified birds, from the standpoint of locomotion, would be the two very different groups of flightless birds, the Ratitæ and the Sphenisci.

In any study of genetic relationships of birds one may find some help in considering the evolution of modes of locomotion.

2. Dimensions of the Canals.

The right ear was the one studied in all cases, and the semicircular canals laid bare by the knife. To measure these curved canals a piece of No. 50 cotton thread was drawn around each and the length marked on it; then the exact length of the thread when straightened out was ascertained. All absolute dimensions are stated in millimeters. All measurements were made twice, at an interval of time, in order to secure the greatest possible accuracy. For units of comparison a median vertical and a median basilar cranial length were employed. The vertical length is the distance from the ventral face of the occipital condyle to the large transverse muscle crest immediately above the foramen magnum; the basilar length is the distance from the posterior face of the condyle to the anterior aperture of the Eustachian tube as marked by bony ridges. These were taken as units of comparison because they can be very accurately measured, but especially because they are lengths of parts of the skull that are perhaps the least subject to variation.

In the tables the first three vertical columns give the absolute lengths of the semicircular canals. The fourth and fifth columns give the absolute basilar and vertical lengths of the skull for comparison. The succeeding three columns show which of the canals are largest. The ninth column gives as the "combined measurement" of comparison the sum of the vertical and basilar cranial lengths divided by two. The tenth column expresses the sum of the absolute lengths of all three canals. And the last column represents this sum divided by the combined cranial measurement given in the ninth column. This last column, accordingly, states the sum of the lengths of the three canals in relation to a definite length of the skull, and may be called the "relative total length."

A comparison of the figures given in the last column of the tables shows what genera have the relatively larger, and what ones the relatively smaller canals, expressed in terms of the cranial measurements. We find then the following associations according to this relative sum total length of the canals:

1.00 mm. to 1.25 mm., the one genus *Pelecanus*.

1.25 mm, to 1.49 mm., *Struthio*, and certain Anatidæ (*Anas*).

Family and Genus.	Length of Anterior.	Length of Posterior.	Length of Exterior.	Basal Measurement.	Vertical Measurement.	Posterior Largest.	Exterior Largest.	Exterior and Posterior Equal.	Combined Measurement.	Sum of Three Canals.	Relative Total Length.
Struthionidæ.											
Struthio camelus Linn.	32	18	14	43	43	×			43	64	1.49
Spheniscidæ.											
Eudytes minor	18	13	11	23	26	×			24	42	1.75
Phalacrocoracidæ.											
Phalacrocorax mexicanus (Brandt.)	12	9	10	16	18		×		17	31	1.82
Pelecanidæ.											
Pelecanus erythrorhynchus Gmel.	17	11	11	31	35			×	33	39	1.18
Ardeidæ.											
Ardea herodias Linn.	19	12	13	18	19		×		18	44	2.44
" *rufescens* Gmel.	12	10	10	15	14			×	14	32	2.28
" " "	15	9	10	15	15		×		15	35	2.33
" " "	12	8	9	12	11.9		×		12	29	2.41
Nycticorax nycticorax nævius (Bodd.)	15	12	13	16	16		×		16	40	2.49
Ciconiidæ.											
Ciconia (? C. alba.)	21	13	12	28	27	×			27	46	1.70
Anatidæ.											
Dafila acuta (Linn.)	11	9	10	19	18		×		18	30	1.66
Aythya americana (Eyt.)	12	9	10	20	20		×		20	31	1.55
Anas boschas Linn.	12	10	9	23	23	×			23	31	1.43
Chen hyperborea Pall	15	8	10	21	20		×		20	33	1.65
Nettion carolinensis (Gmel.)	9	6	7	15	14		×		14	22	1.57
Anser albifrons gambeli (Hartl.)	13	10	10	20	21			×	20	33	1.65
" " " "	13	10	10	20	21			×	20	33	1.65
Querquedula discors (Linn.)	10	7	8	14	14		×		14	25	1.78
Cathartidæ.											
Catharista urubu (Vieill.)	20	11	12	20	23		×		21	43	2.04
Cathartes aura (Linn.)	17	12	12	18	18			×	18	41	2.27
Falconidæ.											
Buteo borealis (Gmel.)	21	17	15	18	20	×			19	53	2.78
Falco columbarius Linn.	15	12	12	10	15			×	12	39	3.25

Family and Genus.	Length of Anterior.	Length of Posterior.	Length of Exterior.	Basal Measurement.	Vertical Measurement.	Posterior Largest.	Exterior Largest.	Exterior and Posterior Equal.	Combined Measurement.	Sum of Three Canals.	Relative Total Length.
Tinamidæ.											
Tinamus brasiliensis	15	11	10	16	12	×			14	36	2.57
Tetraonidæ.											
Colinus virginianus (Linn.)	10	8	9	12	10		×		11	27	2.45
" " "	10	7	8	10	8		×		9	25	2.77
Callipepla squamata (Vig.)	10	7	8	9	9		×		9	25	2.77
" " "	10	7	8	10	9		×		9	25	2.77
" " "	10	7	8	10	9		×		9	25	2.77
Cyrtonyx montezumæ mearnsi (Nels.)	11	9	9	12	9			×	10	29	2.90
Phasianidæ.											
Meleagris gallopavo Linn.	17	13	11	22	21	×			21	41	1.99
Gallus bankiva Linn.	14	12	10	18	10	×			17	36	2.11
Rallidæ.											
Fulica americana Gmel.	14	9	10	14	13		×		13	33	2.53
Rallus	13	9	10	14	13		×		13	32	2.46
Porzana	11	6	7	9	8		×		8	24	3.00
Gruidæ.											
Grus mexicana (Müll.)	18	11	12	19	20		×		19	41	2.15
Charadriidæ.											
Squatarola squatarola (Linn.)	16	10	11	11	12		×		11	37	3.36
" " "	13	10	11	11	12		×		11	34	3.09
Charadrius	12	9	9	9	10			×	9	30	3.33
Ægialitis wilsonia (Ord)	8	7	6	8	9	×			8	21	2.62
Ægialitis vocifera (Linn.)	9	7	7	10	9			×	9	23	2.77
Scolopacidæ.											
Numenius longirostris Wils.	13	8	8	9	10			×	9	29	3.22
Gallinago delicata (Ord.)	13	8	8	9	10			×	9	29	3.22
Tringa maculata (Vieill.)	9	6	7	8	8		×		8	22	2.75
Tringa bairdii (Coues)	9	5	6	9	8		×		8	20	2.50
Ereunetes pusillus (Linn.)	8	5	6	7	6		×		6	19	3.16
" " "	8	5	6	7	6		×		6	19	3.16

Family and Genus.	Length of Anterior.	Length of Posterior.	Length of Exterior.	Basal Measurement.	Vertical Measurement.	Posterior Largest.	Exterior Largest.	Exterior and Posterior Equal.	Combined Measurement.	Sum of Three Canals.	Relative Total Length.
Scolopacidæ.—*Continued.*											
Calidris arenaria (Linn.)	8	6	6	7	7			×	7	20	2.85
Totanus melanoleucus (Gmel.)	12	8	9	11	10		×		10	29	2.90
Totanus flavipes (Gmel.)	11	7	8	9	8		×		8	26	3.25
Symphemia semipalmata inornata (Brewst.)	13	9	9	12	11			×	11	31	2.81
Aphrizidæ.											
Arenaria morinella (Linn.)	11	8	7	9	10	×			9	26	2.88
Laridæ.											
Larus atricilla Linn	16	8	7.5	13	12.3	×			13	31	2.38
Larus delawarensis Ord	15	9	8	14	12	×			13	32	2.46
Sterna maxima Bodd	14	10	8	15	17	×			16	32	2.00
Stercorariidæ.											
Stercorarius longicaudus (Vieill.)	14	10	9	13	14	×			13	33	2.53
Columbidæ.											
Columba fasciata (Say)	14	9	10	12	13		×		12	33	2.91
Columba æneas	14	9	8	12	11	×			11	31	2.81
Turtur auritus	12	7	6	10	9	×			9	25	2.77
Zenaidura macroura (Linn.)	10	6	7	9	9		×		9	23	2.66
Cuculidæ.											
Geococcyx californianus (Less.)	18	8	11.5	14	13		×		13	31	2.84
Psittacidæ.											
Psittacus erythacus	13	10	10	17	15			×	16	33	2.06
Alcedinidæ.											
Ceryle alcyon (Linn.)	13	10	9	12	13	×			12	32	2.66
Strigidæ.											
Strix nyctea Linn	23	18	15	18	17	×			17	56	3.29
Bubonidæ.											
? Asio accipitrinus (Pall.)	15	12	11	15	14	×			14	38	2.71
Caprimulgidæ.											
Phalænoptilus nuttallii nitidus (Brewst.)	11	6	7	9	8		×		8	24	3.00
Chordeiles virginianus (Gmel.)	11	8	8	9	10			×	9	27	3.00

Family and Genus.	Length of Anterior.	Length of Posterior.	Length of Exterior.	Basal Measurement.	Vertical Measurement.	Posterior Largest.	Exterior Largest.	Exterior and Posterior Equal.	Combined Measurement.	Sum of Three Canals.	Relative Total Length.
Caprimulgidæ.—*Continued.*											
Chordeiles acutipennis texensis (Lawr.)	11	8	8	8	9			×	8	27	3.37
Trochilidæ.											
Trochilus colubris (Linn.)	5	4	4	3.8	3			×	3	13	4.33
Picidæ.											
Dryobates villosus harrisi (Aud.)	10	8	9	10	11		×		10	27	2.70
Dryobates pubescens (Linn.)	9	7	7	9	8			×	8	23	2.87
Dryobates scalaris bairdi (Malh.)	10	8	8	9	10			×	9	26	2.88
Melanerpes erythrocephalus Linn.	12	9	10	9	10		×		9	31	3.44
Colaptes auratus luteus Bangs.	13	10	12	13	12		×		12	35	2.91
Tyrannidæ.											
Myiarchus cinerascens (Lawr.)	9	6	7	9	9		×		9	22	2.44
Corvidæ.											
Corvus americanus Aud.	16	11	13	14	15		×		14	40	2.92
Icteridæ.											
Molothrus ater Bodd.	9	6	7	7	7		×		7	22	3.14
Sturnella magna hoopesi Stone	10	6	8	10	10		×		10	24	2.00
Fringillidæ											
Amphispiza bilineata deserticola (Ridgw.)	7	5	6	6	6		×		6	18	3.00
Tanagridæ.											
Piranga rubra (Linn.)	9	7	7	8	8			×	8	23	2.87
Hirundinidæ.											
Progne subis (Linn.)	9	6	5	9	9	×			9	20	2.22
Mniotiltidæ.											
Dendroica coronata (Linn.)	8	4	5	6	6		×		6	17	2.83
Troglodytidæ.											
Galeoscoptes carolinensis (Linn.)	9	6	7	9	9		×		9	22	2.44
Paridæ.											
Parus atricristatus (Cass.)	8	6	6	8	8			×	8	20	2.50
Turdidæ.											
Merula migratoria (Linn.)	10	7	8	9	9		×		9	25	2.77

1.50 mm. to 1.74 mm., the families Ciconiidæ, Anatidæ (*Dafila, Aythya, Chen, Nettion, Anser*).

1.75 mm. to 1.99 mm., the families Spheniscidæ, Phalacrocoracidæ, and Anatidæ (*Querquedula*).

2.00 mm. to 2.22 mm., the families Carthartidæ (*Cartharista*), Gruidæ, Phasianidæ, Laridæ (*Sterna*), Psittacidæ.

2.23 mm. to 2.46 mm., the families Ardeidæ (*Ardea*), Cathartidæ (*Carthartes*), Tetraonidæ (*Colinus*), Laridæ (*Larus*), Tyrannidæ, Hirundinidæ and Troglodytidæ.

2.47 mm. to 2.70 mm., the families Ardeidæ (*Nycticorax*), Tinamidæ, Rallidæ (*Fulica, Rallus*), Charadriidæ (*Ægialites*), Scolapacidæ (*Tringa*), Stercorariidæ, Columbidæ (*Zenaidura*), Alcedinidæ and Paridæ.

2.71 mm. to 2.94 mm., the families Falconidæ (*Buteo*), Picidæ (*Dryobates, Colaptes*), Tetraonidæ (*Colinus, Callipepla, Cyrtonyx*), Charadriidæ (*Ægialites*), Turdidæ, Scolapacidæ (*Tringa, Calidris, Totanus, Symphemia*), Aphrizidæ, Columbidæ (*Columba, Turtur*), Mniotiltidæ, Cuculidæ and Tanagridæ.

2.95 mm. to 3.18 mm., the families Rallidæ (*Porzana*), Charadriidæ (*Squatarola*), Scolapacidæ (*Ereunetes*), Icteridæ, Caprimulgidæ (*Phalænoptilus, Chordeiles*), and Fringillidæ.

3.18 mm. to 3.32 mm., the families Falconidæ (*Falco*), Strigidæ and Scolopacidæ (*Numenius, Gallinago*).

3.32 mm. to 3.56 mm., the families Charadriidæ (*Squatarola, Charadrius*), Caprimulgidæ (*Chordeiles*) and Picidæ (*Melanerpes*).

4.33 mm., the family Trochilidæ.

These data show that birds of the most diverse forms of locomotion, and of very diverse affinities may show the same relative sizes of semicircular canals. For example an excellent flyer, the black vulture (*Catharista*), and the poor flyers, the chicken (*Gallus*) and the parrots (Psittacidæ), have the same relative measurement, 1.98 mm. to 2.22 mm. Further, in certain families the genera may exhibit great differences in these measurements, as particularly in the Charadriidæ and Anatidæ.

The relative sizes of the three semicircular canals to each other may be summed up as follows:

The anterior canal is always the largest.

The posterior canal is larger than the exterior in all the follow-

ing families: Struthionidæ, Spheniscidæ, Ciconiidæ, Tinamidæ, Phasianidæ, Aphrizidæ, Laridæ, Strigidæ, Stercorariidæ, Alcedinidæ, Bubonidæ, and Hirundinidæ.

The exterior canal is larger than the posterior canal in the families: Phalacrocoracidæ, Cuculidæ, Tyrannidæ, Corvidæ, Icteridæ, Fringillidæ, Mniotiltidæ, Troglodytidæ, and Turdidæ.

The posterior and exterior canals are equal in the families: Pelecanidæ, Tanagridæ, and Paridæ.

The posterior canal is larger than the exterior, or the posterior and exterior canals are equal, in the family Falconidæ.

The exterior canal is larger than the posterior canal, or the exterior and posterior canals are equal, in the families; Ardeidæ, Cathartidæ, Tetraonidæ, Caprimulgidæ, and Picidæ.

Falling under none of the above groups are the families: Anatidæ, Charadriidæ, Scolapacidæ, and Columbidæ; in each of which there are great generic differences of the relative sizes of the canals.

3. Types of Canals.

In taking the measurements of the canals, I noticed that the position of the canals with reference to one another or the angle at which they are joined, was not always the same. There are

Fig. 1.

Fig. 2.

Fig. 3.

Fig. 4.

Fig. 5.

five types, though these intergrade, under which all species may be grouped (Figs. 1–5). It will be noticed that these are differences mainly of the anterior canal, depending on its length.

Type I. (Fig. 1), the genera *Struthio* and *Tinamus*.

Type II. (Fig. 2), Cathartidæ, *Geococcyx*, Scolopacidæ, Laridæ, *Stercorarius*, *Columba*, *Zenaidura*, *Ceryle*, Caprimulgidæ, *Strix*, *Asio*, Tetraonidæ.

Intermediate between types III. and V. are the genera *Turtur*, *Melanerpes*, and *Dryobates*.

Type III. (Fig. 3), *Ardea*, *Phalacrocorax*, *Ara*, Anatidæ.

Type IV. (Fig. 4), *Ciconia*, Rallidæ, *Grus*, *Eudytes*, *Psittacus*.

Intermediate between III. and IV. are the genera *Pelecanus*, and *Meleagris*.

Type V. (Fig. 5), *Falco*, Charadriidæ, *Colaptes*, *Trochilus*, and the Passeres.

In the above, as in the results from the comparison of the measurements, the types of canals bear no relation to locomotion; for different genera having very different modes of locomotion have the same type of canal; for example in type II. occurs *Catharista*, one of the best flyers, and in the same group *Geococcyx* which seldom flies but is one of the ablest runners.

4. Eustachian Tube Apertures.

The Eustachian tubes have a common œsophageal opening in all cases, except in the genera *Struthio*, *Eudytes* and *Tinamus*, where there is a pair of apertures.

The Eustachian tube is a perfect bony tube in the following: *Sterna*, *Squatarola*, *Ceryle*, *Psittacus*, *Geococcyx*, Tetraonidæ, Phasianidæ, *Catharista*, *Falco*, *Eudytes*, and *Tinamus*. In all other cases it is imperfectly ossified.

Conclusions.

From a careful consideration of all the preceding data, the following conclusions may be drawn:

1. The anterior canal is always the largest.

2. The exterior canal is larger than the posterior in the greater number of cases.

3. The relative measurements of the canals to each other, as well as their sums, bear no direct relation to modes of locomotion.

4. Relative dimensions certainly stand in no relation to broader racial affinities and such dimensions can be used as taxonomic characters only to limited extent.

5. What occasions differences in these dimensions remains to be determined.

6. The anterior vertical canal is the one most subject to variation of position.

7. Types of bony labyrinths, distinguished according to relative positions of the canals, appear to stand in no relation to mode of locomotion, but to a certain extent are indicative of genetic affinity.

8. There are two main modes in the position of the pharyngeal aperture of the Eustachian tubes: a common pharyngeal aperture, or two distinct ones.

Bibliography of the Osseous Canals of Birds.

None of the following works, except that of Farrar, bear directly upon the question undertaken by me, but the list is given to show what has been done upon the bony canals.

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LONGEVITY OF A VELVET ANT.

ADELE M. FIELDE.

Finding no printed record concerning length of life in the Mutilidæ, I think there may be value in an observation recently made by me upon a single female of *Spheropthalmia occidentalis*.

She was captured in Arizona early in June, 1905, by Professor Edward S. Morse, of Salem, Mass.; reached my hands on the first day of the ensuing August; and lived under my observation until the twelfth of August, 1906. She was kept by me in a small glass ant-nest of the Fielde pattern, in which I fixed three glass vials whose interior diameter was scarcely greater than that of her own abdomen. Into one of these vials she often crept; and she hibernated there during the winter of 1905–6, at a temperature between 65° and 75° F. Whenever the temperature rose as high as 80° F. she awoke, walked about and perhaps ate from the particle of honeyed sponge-cake that always awaited her in the adjoining compartment of the nest, where there was also a bit of sponge that was kept saturated with clean water. In summer she often sought the more humid part of the nest.

She preferred darkness to light; but, like the formicid ants, she avoided only the ultra-violet rays. When sheltered by an orange pane which excluded the ultra-violet rays, she was manifestly unaware that she was not in complete darkness. Like the formicid ants she appeared to be blind to other than the actinic rays.

When first received by me, she once stung me sharply, but thereafter she became acquainted with my hand, seemed to greatly enjoy walking about upon it, and never left it willingly except to enter her nest.

I never heard her stridulate when in the nest; but whenever I enclosed her in the warm darkness of my fist, she sounded her charming, harmonious notes, representing but two keys. Probably each key was determined by one of the two overlapping sclerites on the top of the abdomen. Great variation in the

rapidity of the muscular contraction produced at different times an effect like that of playing different tunes on a minute stringed instrument. The sound was sometimes audible at a distance of one yard; but it was much more often audible only when the fist inclosing the insect was in contact with the ear. Occasionally she stridulated continuously for as long a time as three or four minutes.

It is probable that this velvet ant lived at least three summers. The tendency to hibernate in a snug, arid cell, at temperatures below 75° F.; makes it unlikely that her progenitors had reared her in the summer in which she was captured. She must, then, have been hatched as early as the summer of 1904. Her existence was probably shortened by an unnatural environment. My observation of this specimen therefore indicates that the female of the velvet ant may live several years.

THE DEGENERATE EYES IN THE CUBAN CAVE SHRIMP, PALÆMONETES EIGENMANI HAY.[1]

FRANK H. PIKE.

The blind shrimp whose eyes are considered in this paper is common in the pools of the sink holes and caves near Cañas on the Western Railway of Cuba. The material was collected by Professor C. H. Eigenmann, who described the localities in his account of "The Fresh-water Fishes of Western Cuba" ('03). The species was described by W. P. Hay, in his paper "On a Small Collection of Crustaceans from the Island of Cuba" ('03).

Material. — Four specimens were available for study. The general topographic relations were determined from a surface study of the different specimens. The histological detail was worked out mainly from one series of sections. The normal marine shrimp from Wareham, Mass., was used for comparison.

Methods. — The animals were killed in formalin and transferred to seventy per cent. alcohol. Preparatory to sectioning, the head was cut off and placed in Perenyi's fluid for about twenty-four hours, dehydrated, cleared in xylol, and imbedded in paraffin. Longitudinal horizontal sections were made. They were mounted on the slide by the water method, and stained with hæmalum and eosin. One specimen was killed in Vom Rath's fluid and stained with safranin. The eyes of the normal shrimp were depigmented by being placed in a ten per cent. solution of nitric acid for from twenty-four to seventy-two hours before being placed in Perenyi's fluid. The remainder of the technique was the same as for the degenerate eye.

[1] Contributions from the Zoölogical Laboratory of Indiana University, No. 58. This study was completed many months ago. Its publication has been delayed to secure good photographs of the critical sections. The material was collected with a grant from the Carnegie Institution.

The Gross Anatomy.

General External Appearance. — Like the cave crustaceans of southern Indiana and Kentucky, the Cuban shrimp is clear white or colorless. The antennæ are extremely long and the chelæ are relatively longer than in the out-door or normal species. The eye-stalks are plainly visible but not as prominent as in the normal species. In the cave-crayfish from southern Indiana and Kentucky the eye-stalks are almost hidden beneath the rostrum, when viewed from above. They are much more prominent in the present species.

Surface Views of the Eye-stalks. — I. Viewed from the side, with moderate magnification, the eye-stalks are seen projecting forward from the anterior lateral part of the head just below and to one side of the rostrum (Fig. 1). The eye-stalk is not termi-

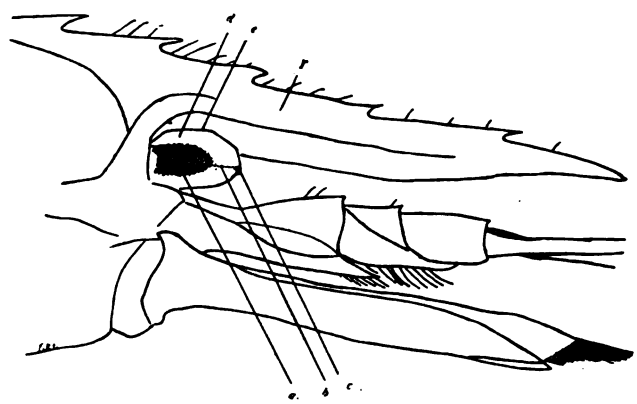

FIG. 1. Eye-stalk viewed from side. *r*, rostrum; *e*, eye-stalk; *a*, optic ganglion; *b*, optic nerve; *c*, retina; *d*, space filled with hæmolymph.

nated by a hemispherical cap as in the normal species, but by a peculiarly shaped, somewhat acute-tipped cone. The stalk is directed upward, forming a small angle with the horizontal. If the line drawn from the middle point of the base through the tip be taken as the long axis of the eye, the part of the stalk above this line will be at all points convex in outline. Beginning at

the tip, the part of the stalk below this line is slightly concave for about one-half the distance from the tip to the base of the cone. From this middle point of the base of the cone on to the base of the stalk, the outline is again convex.

The cuticula is clear white and partially transparent, with no trace of facets in the corneal region. There may be seen through it (*a*) a white mass, very nearly concentric in curvature with the outline of the eye-stalk, extending from the base forward a little more than two-thirds of the distance to the tip of the stalk, and terminating in a blunt cone; (*b*) a find strand running from a point a little below the end of this cone forward to (*c*) a small group of granules in the tip of the stalk. These granules appear to be spread over a small area on the proximal face of the cuticular tip, and taper to a narrower diameter where the thread joins them; (*d*) a space appears between the white mass (*a*) and the cuticula on the sides of the stalk. No trace of pigment is visible with any magnification. In sections these structures are seen to to be (1) the optic ganglion, (2) the optic nerve, (3) the remnants of the retina and dioptric apparatus, and (4) a space filled with hæmolymph, respectively.

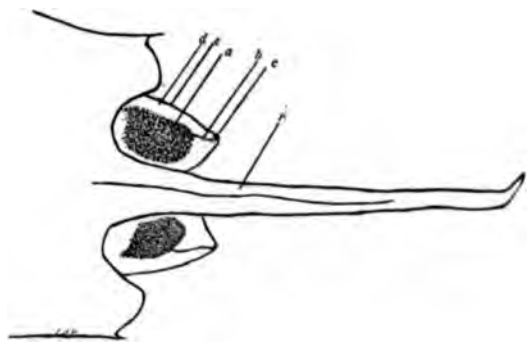

FIG. 2. Eye-stalk viewed from above. *r*, rostrum; *e*, eye-stalk; *a* optic ganglion; *b*, optic nerve; *c*, retina; *d*, space filled with hæmolymph.

Any great amount of shrinkage due to reagents would certainly break the fine strand of fibers running to the granules in the tip. I am inclined, therefore, to believe that the greater part of the space seen between the optic ganglion and the cuticula is normal in this eye.

II. Viewed from above, the outer side of the eye-stalk slopes inward toward the rostrum at an angle of 15 to 20 degrees to the longitudinal axis of the body. (Fig. 2.) The stalk terminates in a blunt, slightly rounded cone. The anterior mesial

edge of the stalk slopes inward and backward to the rostrum. The entire outline of the eye-stalk, when viewed from above, is singularly free from the graceful curvature of the normal eye-stalk. The same structures seen in the side view appear in this view. The tip of the eye-stalk is directed outwards away from the rostrum, and the optic nerve springs from the outer side of the anterior end of the optic ganglion. The granules are applied to the lateral surface of the stalk. The anterior end of the optic ganglion is more rounded than it is in the side view.

General Appearance in Sections. — The specimen on which the account of the minute anatomy here recorded is based, measured 17 mm. in length. The eye-stalk is 1,044 micra in its greatest length and 793 micra in its greatest width. The ratio of the length of the eye-stalk to its width is 1.3. The cuticula of the eye-stalk varies in thickness. It is 14.3 micra thick on the outer side of the stalk away from the rostrum, 10.9 micra in the region where the retinal elements are applied to it, and 7.2 micra on the inner side of the tip next the rostrum.

FIG. 3. Low power microphotograph of eyes of blind shrimp from above. *ct*, corneal cuticula; *c*, retina; *a*, fibrous portion of optic ganglion; *o*, cellular portion of optic ganglion; *h*, hypodermis.

In the left eye, the hypodermis and the retina have adhered to the tip of the stalk, but the optic nerve has broken so that the retina and the optic ganglion are separated. The retina is applied to the side of the eye-stalk. In the right eye, the hypodermis and the retina have pulled loose from the corneal cuticula, but the optic nerve is unbroken. (Fig. 3.)

The optic ganglion appears as a fibrous area surrounded by small cells with large, deeply-staining nuclei. The hypodermis has been torn loose from the sides of the eye-stalk and, in places, lies close to the optic ganglion. Transverse sections of the eyes of a specimen killed in Vom Rath's fluid show the hypodermis lying in contact with the cuticular wall.

In the right eye, a fibrous strand — the optic nerve — extends from the anterior outer part of the ganglion forward to the retinal elements. The optic nerve is bounded on each side by a layer of pavement cells with prominent nuclei. (Fig. 5.) In the right eye, the retinal elements are spread out rather loosely in the form of a fan, the apex of which is directed toward the optic nerve. Some spongy tissue, probably coagulated hæmolymph, appears on each side of the optic nerve between the bounding membrane and the hypodermis.

FIG. 4. Eye of normal shrimp; same magnification as Fig. 3.

Comparison of Normal and Degenerate Eyes. — Fig. 3 is from a photograph of the eyes of the blind shrimp. Fig. 4 is from a photograph of the author's preparation of a normal shrimp eye, made with the same magnification used in Fig. 3. It will be noticed (1) that the degenerate eye is much smaller than the normal eye, (2) that its optic ganglion is apparently a single mass of cells and fibers, and is not divided into parts as in the normal eye, and (3) that the retina and dioptric apparatus are represented by the merest vestiges of these structures as they exist in the normal eye.

Fig. 5. Eye of blind shrimp. 4 mm. objective and 4 projection eye-piece. *rt*, retinula cells; *b*, optic nerve; *mb. l*, bounding membrane of optic nerve; *h*, hypodermis; *ct*; corneal cuticula; *d*, coagulated hæmolymph.

A few numerical results will make these contrasts more evident. There are present in each eye of a normal decapod about 2,500 ommatidia. (Parker, '95). Each ommatidium is composed of sixteen elements: two corneagen cells, four cone cells, two distal retinula cells, one rudimentary and seven functional proximal retinula cells, and one or more accessory pigment cells. In the retina of a normal eye there are, then, about 40,000 cells, about 22,000 of these being retinula cells. There are in the degenerate eye not more than 100 and not less than 50 retinula cells, and not more than ten or twelve cone cells. The cone cells in the normal eye outnumber those in the degenerate eye a thousand to one, and the retinula cells of the normal eye outnumber those of the degenerate eye by at least two hundred to one.

As stated above, the ratio of the length of the eye-stalk to its width in the degenerate eye is 1.3. In a normal crayfish eye I found this ratio to be 1.9. In the eye shown in Fig. 4, this ratio is 1.6. I do not know the range of variation in this ratio in the normal eye, but I feel safe in saying that the degenerate eye has decreased relatively more in length than in width.

The Minute Anatomy.

The Retina in the Left Eye. — The hypodermis consists of oblong cells closely applied to the cuticula and containing relatively large nuclei, Fig. 5. At the extreme tip, the cells become more spherical in shape and are slightly separated from the cuticula. The nuclei are less prominent than in the other hypodermis cells. Beneath these smaller cells is a layer of three somewhat larger spherical cells. Below these occurs a layer of two cells. All have small nuclei and clear cytoplasm. Two irregular masses, staining deeply with hæmalum but exhibiting no discoverable structure, lie, one on each side, immediately beneath the hypodermis and to one side of the clear cells above mentioned. Immediately beneath these dark masses are two other irregular, structureless patches staining diffusely with eosin and not at all with hæmalum.

Miss Seaton ('03) states that, in the compound eyes of Machilis, the cone cells do not stain readily with plasma stains. In some of my own preparations, the cone cells do not stain as readily

with a plasma stain as do some of the other tissues of the eye. Does this consideration, together with the position of these clear cells, afford us sufficient evidence to warrant us in regarding them as degenerate cone cells? I shall presently give a stronger argument in support of this conclusion. What the dark masses and the irregular patches of tissue represent I am not prepared to say. I am not certain that they posses any special morphological significance.

Beneath these structures is a group of larger cells with large, deeply staining nuclei. The nucleus occupies nearly all the space in the cell, there being only a narrow ring of cytoplasm within the cell wall. A plainly marked fibrous tract, the optic nerve, extends from these cells toward the optic ganglion. A fine membrane separates this fiber and cell tract from the hæmolymph or spongy tissue on either side. The large cells are, as I shall presently show, retinula cells.

The Retina in the Right Eye. — The tearing loose of the retina from the cuticula has destroyed the cone cells and the hypodermis at the tip so that no trace of either is visible. The looser arrangement of the large, deeply staining cells permits of a more accurate determination of their relation to the fibers of the optic nerve. With the oil immersion lens, the fibers can be traced up to these cells and can be seen ending in them. (Figs. 6, 7, *n* and *rt.*)

The objection may be urged that, since hæmalum is not a nerve stain, the fibers in question have not been shown to be nerve fibers. The reaction to iron hæmatoxylin is not as marked as in the fibers of the cephalic ganglion, but is still marked enough to indicate that they are nerve fibers. The strongest evidence that they are nerve fibers is their relation to the optic ganglion and their correspondence in position and relation to the nerve fibers in the normal eye. I believe that the simplest explanation open to us is that the fibrous strand represents the optic nerve.

Parker ('95) states that, in the normal decapod (*Astacus*) eye, the fibers of the optic nerve pass through the retinula cells and disappear in the region of the rhabdome. There is no instance known to me in which degeneration has caused a change in the

location of a nerve ending. I consider the deeply staining cells to be degenerate retinula cells. The cone cells have lost their distinctive characteristics much more than the retinula cells.

Summary. — The degenerate eye has a relatively shorter stalk than the normal eye. The corneal cuticula is thinner than that on the outer side of the eye-stalk. The optic ganglion appar-

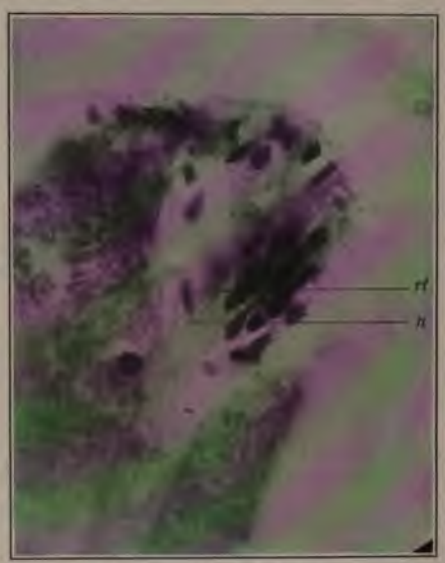

FIG. 6. Eye of blind shrimp. 2 mm. oil immersion objective, 4 projection eye-piece. x 1,000. *rt*, retinula cell in which a nerve fiber, *n*, may be seen to end.

ently consists of a single mass of fibers and cells. There is normally some space between the optic ganglion and the cuticula of the eye-stalk. The optic nerve, extending from the optic ganglion to the retina, is present. The retinula cells, in which the fibers of the optic nerve terminate, still persist. The vestiges of the cone cells may be, and probably are, present. The structures serving to distinguish light from darkness, probably being older phylogenetically than the structures concerned with the production of a definite image, are less degenerate than the latter. The active structures of the eye, represented by the retinula cells, have degenerated vastly more than the passive structures such as the cuticula.

Several questions arise to which no satisfactory answer can at present be given. (1) To what extent has the internal morphology of the neurones of the adult optic ganglion been modified by degeneration? (2) To what extent has the degeneration of the axones affected the cephalic ganglion of the animal? (3) What is the condition of the eyes in the embryo? The neurones of the

FIG. 7. Same eye as in Fig. 6, but different focus. x 1,000. *n*, a nerve fiber ending in *rt*, a retinula cell; *d*, coagulated hæmolymph; *mb*, bounding membrane of optic nerve; *b*, fiber of optic nerve; *h*, hypodermis; *ct*, corneal cuticula.

Microphotographs by Dr. D. W. Dennis, Earlham College, Richmond, Indiana.

optic ganglion seem to have degenerated so much that their internal morphology is enigmatical. No nuclear wall nor chromatin threads could be seen in any of them, even when stained with iron hæmatoxylin. It is an open question what the phylogeny of the decapod eye has been, and what bearing the present case of degeneration may have on the Law of Biogenesis. The embryological history might throw some light on this latter phase of the question, but it has been impossible so far to find embryos.

I wish to acknowledge the help received from the sources indicated in the bibliography. I am under obligations to Professor Eigenmann and to Dr. W. J. Moenkhaus for many suggestions and aid in completing the work. Professor Donaldson and Dr. Hatai of the University of Chicago have given valuable suggestions.

ZOÖLOGICAL LABORATORY,
INDIANA UNIVERSITY, August, 1904.

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BIOLOGICAL BULLETIN

THE HISTOLYSIS OF THE MUSCULATURE OF CULEX PUNGENS DURING METAMORPHOSIS.[1]

FRANCIS A. HULST, M.D.

INTRODUCTION.

The phenomena attending the metamorphism of insects has attracted the attention of biologists for many years. The marked contrast which the larval form bears to the adult insect was alone sufficient, at first, to attract the observation of naturalists. Since Metschnikoff's work in 1883 setting forth the theory of phagocytosis, much has been done to determine the role of the phagocytes in the development of organisms, especially such as exhibit a transformation of form and function in their ontogenetic processes, after leaving the egg and entering upon an independent life. But in spite of much and careful study certain phases of the problem of metamorphosis are far from solved. It is the purpose of this paper to set forth the cytological phenomena associated with metamorphosis as found in *Culex pungens*, paying particular attention to phagocytosis in relation to the histolysis of the musculature. The work was done during the winters of 1902–'03 and 1903–'04 in the Zoölogical Laboratories of Syracuse University, under the directon of Dr. Chas. W. Hargitt, whose supervision of the work, as well as the valuable aid and suggestions of Dr. Smallwood, are gratefully acknowledged.

METHODS.

Material was collected during the summer months of 1902 and 1903 and fixed by several methods. Picro-sulphuric acid, corrosive sublimate and Zenker's fluid gave good results. It

[1] Contributions from the Zoölogical Laboratory, Syracuse University.

was found necessary, to insure good fixation, to puncture the chitinous integument before immersing in the respective fluids. Specimens punctured, or clipped in two or three different places with fine-pointed scissors, and then plunged at once into hot 35 per cent. alcohol gave very good results. Specimens killed and preserved in formalin gave poor fixation, and those killed in cold alcohol were also inferior to the above.

Serial sections cut three microns and stained on the slide by the iron-hæmatoxylin method were made and studied in the various stages of development. Bordeaux-red made an excellent counter-stain for these sections. To supplement these, series were stained in Delafield's hæmatoxylin and eosin.

Historical.

Although the subject of metamorphism has excited the interest of naturalists of all ages, for definite work upon the problem of the internal phenomena, one need go no further back than the work of Weismann in 1864; while it was that of Metschnikoff ('83), introducing the idea of phagocytosis, that gave the incentive for closer and more thorough investigation of the subject.

Weismann studied the postembryonic development of *Musca vomitoria* and called attention to the degeneration of the tissues of the pupa. He introduced the term "histolysis" to indicate the process of change which took place. According to him the degeneration is of a fatty nature and the resulting mass mingles with the blood whose elements have also degenerated forming altogether a thick fluid (Brei). Fragments of the mass become isolated, surround themselves with a membrane, take on a granular appearance, lose their fatty particles and acquire a nucleus. These conglomerates of detritus were termed granular spheres (Körnchenkügeln), and to them he attributes the formation of free cells *de novo*, and believes they furnish material for the construction of new tissues.

Ganin ('77) studied the muscidæ and agrees largely with Weismann. He believes that the products of degeneration serve in the formation of new organs only as nutritive substance. He demonstrated the existence and composition of the imaginal discs, which persist as embryonic rudiments in the various organs

through the growing stage, and are the centers of new growth during the process of pupation.

Viallanes ('82) describes the destruction of the fat-body, in which he finds a setting free of granules by rupture of the cell membrane. These granules constitute a sphere stained by carmine surrounded by a non-staining substance, and having a distinct outline resembling protoplasm, and are, probably, daughter-cells of the adipose cells. They resemble the embryonic cellules which constitute the outline of the first muscles of flight in the imago. The granular spheres of Weismann, Viallanes divides into two classes, the large and the small, having many or few nuclei respectively. The latter may tend to the formation of a fatty fluid (purée) on dissolution of the elements. The large ones are hypertrophied adipose cells and include the embryonic cellules.

In muscular destruction he speaks of a "regressive evolution" and "evolution by degeneration." In the former the muscle nuclei become spherical, enveloped by protoplasm and proliferate. The contractile substance disappears as if nourishing the new elements, an area of granules resulting. In the latter the nucleoli first disappear followed by a dissolution of the contractile substance.

As already stated, Metschnikoff ('83) changed the course of study in these lines by the discovery of phagocytosis. He first described certain cells of the animal body as scavengers, and gave to them the name "phagocytes." Since then much of the work done on the problem of metamorphosis has been upon this subject. Metschnikoff describes muscular phagocytes which play a part in the destruction of the muscles in the metamorphosis of insects and tad-poles. This phagocyte has for its nucleus that of a muscle fiber which becomes enlarged and surrounded by a granular protoplasm, finally breaking loose from the muscle and ingesting fragments of tissue.

Following Metschnikoff, Kowalevsky ('85) took up the work. He finds leucocytes, penetrating into the muscular substance of *Musca vomitoria* a few hours after passing into the nymphal stage, and, by their pseudopods, tearing the tissues and breaking passages for others. After incorporating fragments the leucocytes return to the circulation swollen with the ingested particles. These, Kowalevsky believes, are the same as the granular spheres

of Weismann. The nuclei of the muscles resist longer the agents of destruction and may be seen in the fragments of contractile tissue carried off, but are finally destroyed by the leucocytes and undergo fatty degeneration. At the end of two days all the leucocytes are converted into these granular spheres but continue to ingest fragments of destroyed tissue.

Originally no granular spheres are found in the young larva but the tissues are surrounded by fluid containing blood cells in large numbers. In pupæ of one or two hours there is a penetration of the blood cells into the muscle substance beginning at the head end of the body. They surround the bundles and some passing within the sarcolemma lie flat between the latter and the muscular substance, but soon send out prolongations which divide the muscles into bits. These same blood cells then ingest the fragments. After the muscles of the cephalic portion of the body have been attacked in this manner, the adipose body is the seat of a similar change. Kowalevsky has seen granules break through the surface of the salivary gland, and others coming to join them, give the appearance of a morula. At the end of forty-eight hours he saw the number of granular spheres on the surface diminish, and attributes the fact of this diminution to their penetration into the adipose cells, a process different from that found by Viallanes ('82).

Korotneff ('92) studied Tinea (*Trichonsis tonsurans*) where he finds the following to be the chief phases of metamorphosis. There are no spherical mesenchyme cells in the larva, the cœlome contains only leucocytes and granular spheres. The leucocytes take absolutely no part in the degeneration of the tissues. The formation of the imaginal muscles is considered as a reformation of the larval muscles. The fibrillar portion of the muscles becomes granular and contracted and the nuclei multiply, chiefly on one side. The granular bundles are resorbed or undergo a slow dissolution without the leucocytes taking any part in the change, while the nuclear bundle parallel to it moves off from the surface and soon produces new fibrillæ. On the other hand, he believes in the intervention of leucocytes in the histolysis of *Musca vomitoria*, for the more energetic phenomena necessitate a rapid transformation of the tissular detritus into new organs.

He compares the two modes to those which pass in chronic and acute pathological phenomena.

Bataillon ('90) found that the muscles destined to disappear in the tail of the pollywog (anoures) presented undeniable evidence of degeneration before the appearance of the phagocytes. Phagocytes seem drawn toward the place of degeneration, but not before many sarcolytes (muscular fragments, containing nuclei or not) are present, and these latter may break up without being incorporated in the phagocytes. Thus the migratory cells play only an accessory part in the process. Metschnikoff ('92) takes up the subject again and maintains in response to Bataillon, that in the muscles there is shown neither a disappearance of the nuclei, spontaneous degeneration of the muscular fasciculus, nor infiltration of the muscles by leucocytes. The atrophy then is brought about by muscular phagocytes derived from the muscular nuclei which persist and are surrounded by a granular sarcoplasm. Thus the muscular fasciculus is disassociated and absorbed by one of the elements which constitute it without any intervention on the part of the leucocytes. "Neither the disassociation of filaments nor the formation of myoplastic fragments or sarcolytes are ever spontaneously produced without the active coöperation of the muscular phagocytes." [1]

Bruyne ('98), in an admirable paper, gave a detailed account of a thorough study of the metamorphosis of *Musca vomitoria*, as well as other insects and crustaceans. He finds the muscles reduced to isolated fasciculi in which the striation is distinctly recognizable. The nuclei are hypertrophied and lodged in finely granular protoplasm. Part of the muscle bundle retains its morphological characteristics and stains with hæmatoxylin; other parts show the above phenomena. Furthermore, numerous sarcolytic fragments taking the rose tint of the eosin are found in the excavations of the muscles and about the bundle; also small ones shading in color to a pale violet. These manifestly mark a transition from the normal muscular bundle to the necrotic sarcolytes. Leucocytes do not participate in these changes. The destruction of the muscles, which is evident, has come about then

[1] "Response a la critique de M. Bataillon au sujet de l'atrophie musculaire chez les tetard," *Ann. Institut Pasteur*, 1892, p. 236.

without their intervention and before their arrival, contrary to Kowalevsky.

At the end of physiological activity of the muscles, there is a weakening and chemical alteration shown by the staining properties and minute structure, the cause of which is in the muscle itself. The chemical alteration now attracts leucocytes by chemotaxis and these ingest the sarcolytes. At a later period phagocytic leucocytes, empty or gorged, and the fragments upon which they prey, occupy the cavities of the body, and the author believes that the engorged leucocytes are identical with the granular spheres of Weismann. It is the function of these phagocytes to remove the muscular detritus from its place of origin, digest it, and transport the products of this digestion to the places where they may be utilized anew in the animal economy.

Bruyne further notes that the removal and placing in reserve of muscular debris often happens in an entirely different manner, and here he agrees with the views of Metschnikoff. In one case there was no degeneration of the nuclei followed by their incorporation in phagocytes, but on the contrary they were seen to hypertrophy before a trace of degeneration was present. The nucleus with the sarcoplasm around it isolates itself from the rest of the muscle fiber and forms a complete cell. These cells conduct themselves like the muscular phagocytes of Metschnikoff, in the larval tail of the frog, *i. e.*, acting like an amœboid cell, it ingests muscular debris. He calls them " sarcoclasts " or " myoclasts." These cells are numerous, consisting of muscular nuclei surrounded by cytoplasm which radiates in all directions to the periphery and results in a general network in the meshes of which are located the sarcolytes, the largest of which are grouped near the nucleus and retain their rose tint; the smaller, more wasted ones near the periphery show a pale staining property, which facts indicate that there is a change going on within the cell.

In *Bombyx mori* the same author describes the same phenomena as taking place. Both leucocytes and sarcoplastic phagocytes were found, the latter in a relatively small number. Similar conditions were found in other organisms studied by him.

Anglas ('00), studying the wasp and bee, found the muscles to be overrun with leucocytes as soon as the contractile property

was diminished. If present before they are in limited numbers. The phagocytic activity begins only with the physiological and chemical regression of the muscles. Morphologically there is no change, but the inertness permits us to say that it is chemically modified. The intervention of leucocytes is variable in organs of the same type, and it is evident that the cells are not indispensable.

The phenomena attending the dissolution of the fat body are not those of phagocytosis, but a digestive agent is produced which acts upon it. He terms this "lyocytosis" but leaves the mode of the process undefined, other than saying that "lyocytosis is a digestive action of a lyocyte on a cell element which, as a result, enters into cytolysis and becomes a cytolyte."[1] Later he explains in another paper, a digestion taking place by an extracellular process by means of a diastase. This is purely a chemical action brought about by a lyocytic action of neighboring tissues, but whose agents are not easy to determine.[2]

In ants, according to Perez ('00), leucocytes penetrate between the sarcolemma and myoplasm, starting about the nucleus, then between fibrils along the line of least resistance, *i. e.*, there is a leucocytic phagocytosis. Terre ('00), studying the same animals, as well as wasps, finds no such phenomena as Perez describes. He believes it impossible to make leucocytic phagocytosis play a part in the muscular degeneration. Myoblasts were described as found, and these cells and their position resemble what Perez describes as leucocytes. He gives account of two such: (1) embedded in myoplasm, (2) small ones variable in position, usually superficial, sometimes near the larger ones, and hard to determine whether or not surrounded by protoplasm. The small nuclei are found at an early period before there is the slightest evidence of the beginning of metamorphosis. In a later work of the same year he tends to agree with the idea of Anglas and states that metamorphosis may be accomplished without phagocytosis as generally understood. If the term is taken in its literal meaning of ingestion of bodies, it does not always apply, for regression of muscles and other organs may take place by an extracellular digestion. Again he says ('98) that by contact of the small

[1] "Note préliminaire sur les metamorphoses internes de la Guipe et de l'Abeille — la Lycytose," *Compt. Rendu Soc. Biol.*, LII., p. 94.

[2] "Sur la signification des termes phagocytose et lyocytose," *Ibid.*, LII., p. 219.

myoblasts the contractile substance seems to disappear as if by digestion and absorption.

Caullery and Mesnil ('00) claim a phagocytosis in the metamorphosis of crustaceans. The leucocytes arrive outside of the muscular substance at a time when there is a degeneration in the muscles as shown by the indistinctness of the striation. The myoplasm passes into a formless mass of remnants of former muscles in the meshes of a protoplasmic network in which state the nuclei resemble amœbocytes.

Needham ('00) in studying the metamorphosis of the flag-weevil (*Mononychus vuplieculu*) finds an interesting point in the fact that phagocytes do not appear in the destruction of larval tissues until the imaginal stage is entered upon. Then they appear in large numbers in the midst of the fat along the sides of the abdomen. The significance of this is more marked when one considers that the flag-weevil has a complete and rapid metamorphism.

Breed ('03) gives an account of the muscular changes in *Thymalus marginicollis* Chevr. Three groups of muscles receive separate consideration here. One strictly larval, which completely disintegrates and is lost; a second which metamorphose into imaginal muscles; and a third which are strictly imaginal, having no counterpart in the larva. These classes present different characteristics, and the muscles of one class present great individual variation. In the muscles of the second class there is a longitudinal division of the original fibers into several fibers. A destruction of the fibrillæ follows until the muscle fiber becomes a structureless mass of sarcoplasm. The number of nuclei is greatly increased by amitotic division. At an early stage cells derived from the intracellular tracheoles are found between the fibers, which eventually give rise to the new tracheoles. In the muscles of the first class he makes two divisions, one of which undergoes a progressive atrophy with no change of the nuclei until the last stages when they undergo a typical chromatolysis; in the other there is a process similar to that described above for muscles of the second class, *i. e.*, the muscles undergo a change similar to those of the metamorphosing muscles until the stage of reconstruction begins. *Tracheal* cells are found in both of these cases, more especially in the latter, which the author believes

disappear into the blood stream and become imaginal leucocytes. He says "the degeneration of the larval muscles is entirely chemical, there being no evidence of phagocytosis." [1]

Muscular Degeneration in Culex.

In the study of the phenomena attending the postembryonic development, or metamorphosis, of *Culex*, one should first consider certain facts in the life history of the insect. Metamorphosis is complete — the insect belonging to the holometabola. Its larval life has as average duration of nine to ten days, and it passes the pupal stage in three days. This, however, is not constant and one cannot judge the stage of development of a larva or pupa by the number of days elapsed since emergence from the egg. In isolating a group of eggs in a dish of water one finds pupæ at the surface as early as the seventh or eighth day, perhaps, while not all will have reached that stage of their existence until several days later. One then can only judge from the size of the larva as to its stage of development.

The outward manifestations of metamorphism throw little light upon the internal phenomena attending such change. Several moults may mark the steps of development through the larval and pupal life of an insect and the periods of time between such ecdyses show no change in the external morphology though the internal changes are constantly going on. This fact is well accentuated in *Culex*, for while the pupa is looked upon as being the stage of transition from the larva to the imago, the histolysis of the tissues does not correspond to this period. Notwithstanding the fact that metamorphism is complete and rapid, *Culex*, unlike the holometabolic insects so far studied, does not go into a resting stage at the end of larval growth. The pupa is very active. What then shall be expected? Certainly the larval muscles cannot all degenerate and be destroyed during the pupal stage if the insect still is moving its body through the function of these organs. Is there first a new growth of muscles for the imago before the degeneration of the larval fibers? If such were the case we should look for the phenomena of degeneration in the imago and not in the pupa.

[1] "The Changes which Occur in the Muscles of the Beetle During Metamorphosis," *Bull. of Mus. of Comp. Zoöl. Harvard*, Vol. XL., No. 7, p. 373.

These questions are all answerable to a greater or less degree after a study of sections of wrigglers from the time they are two thirds grown until they are ready to emerge. Degeneration begins as early as the two thirds stage and is found first in certain muscles of the thorax. The next are those of the head, and lastly in the abdomen extending distally from the thorax in order. The appearance of imaginal organs is also made in the larva, the thoracic appendages and their muscles beginning to form before the advent of the pupal stage. Thus in one series of sections one may study the degeneration of larval muscles and the regeneration of the imaginal ones. Destruction is not complete in the abdominal segments even in late pupal life, and it is possible, indeed probable that this is completed in the adult insect.

As to the initial cause of the degeneration of the larval muscles, it has been shown that authors disagree. Kowalevsky has made the attacking leucocytes or blood phagocytes (hæmatophages) directly responsible for the disintegration, claiming that they surround the muscle bundle, insert pseudopods into the sarcolemma and so break their way into the substance. The fibers are broken up in this manner and the fragments ingested by the hæmatophages. Bruyne, on the other hand, while admitting phagocytic leucocytosis as an important factor in the disintegration of muscles destined to disappear, does not find them the initial cause of the phenomena. He affirms that muscles lose their striations, become fragmented and that the nuclei hypertrophy before there is an appearance of leucocytes. He believes the cause is to be looked for in the muscles themselves, which degenerate at the end of physiological activity.

It seems indeed probable that, metamorphosis having been established in a group of insects, the phenomena of degeneration, or at least of atrophy, of the organs of the adaptive life, which are not to serve the adult, should occur when the limits of that stage of the life are reached and such organs become inactive. In such a case these organs may be looked upon as foreign bodies and the phagocytes performing their accustomed office, set about their removal. This seems the more probable since the degenerating tissue would attract leucocytes by chemotaxis. This must be modified somewhat in the case of *Culex*, in which

there is no resting stage. Does the cessation of physiological activity mark the beginning of degeneration here? It cannot be otherwise; the muscles which first disappear are those of the head and thorax which would be of as little use to the pupa as though there were a true resting stage; the other muscles are not lost until late. Any muscle must certainly have ended its physiological function before it is removed by what can be considered a normal process.

Further generalizations will be deferred until the conditions observed in *Culex* have been described. The muscles of the larva, before degeneration has begun, show the usual characteristics of muscular tissue in other insects. Bundles of coarsely striated fibers, each with its sarcolemma and nuclei, are arranged longitudinally along the segments of the body. The nuclei are oval and lie close to the contractile substance within the sarcolemma. Immediately surrounding it is a small amount of finely granular protoplasm. With the onset of regressive change in these muscles, there is an increase in the amount of the granular material about the nucleus and the latter becomes separated from the contractile substance, loses its oval shape and hypertrophies. This takes place before there is any appreciable change in the contractile substance. Later the striations are less distinct and become gradually lost, while at the same time the fibers become divided longitudinally into fibrillæ. The sheath of the bundle is stripped away from the fibers by an increasing amount of granular material, in which the hypertrophied nuclei come to lie. All this takes place without the appearance from other sources, or formation from preëxisting elements in the muscles, of active cells of any kind.

From this stage of regression several phases of degeneration present themselves, none of which are constant, nor confined to any one set of muscles. The nuclei lying free in a mass of granular material continue to hypertrophy. The chromatin threads are early lost, and small deep-staining granules appear in the nuclear substance which later become arranged about the periphery close to the membrane. The nucleolus disappears at this time, and the center of the nucleus may be almost transparent. Finally the membrane ruptures and the granules become scattered

in the general tissue detritus. In some cases there is evidently a limited multiplication of the nuclei by direct division at the beginning of degeneration but the products of such a division pursue a course of disintegration not unlike that just described. In fact no direct evidence of such a division can be here given except the relatively large numbers of nuclei found in some instances at an early stage of regression, and all of these come to the same termination.

Along with this degeneration and disappearance of the nuclei there are changes in the contractile substance. The ensheathing layer of the bundle becomes separated and often broken by the increase of the granular material in which the nuclei lie. The fibers become reduced to fibrillæ and fragmentation and dissolution follow. Often in the same material with the nuclei between the outer sheath and the fibers are seen small round cells. These may make their appearance before the striation of the muscles is lost, but never before there is an abundance of the granular material outside of the bundle and never before the nuclei are hypertrophied. They have never been seen to attack the fibers. They have, undoubtedly, a phagocytic function as will be shown, but play a secondary part in the process, for degeneration is well marked before their appearance.

The place of origin of these small round cells is indicated by such conditions as are shown in Plate X., Fig. 4. In the connective tissue surrounding and lying near the muscle are found cells of an elongate form, often overlooked in the fine threads. Though a division of these cells has not been actually observed, a transition from the elongate cell lying between the fibers of connective tissue, to the round cell in the granular material about the muscle seems quite probable. Such cells engage themselves with the ingestion and removal of the products of degeneration, about the muscle bundle, and later they are seen filling the body cavity, in whole or in part, much swollen with particles showing various stages of degeneration.

In other instances such phagocytes are not found at all. There is a regression of the muscles to fibrillæ and a limited amount of the granular material is found. The muscular substance then seems to disappear as if dissolved away by some chemical action.

Here the sheath about the bundle, not being stretched away or broken by the increase of material within, remains longer than the contractile substance showing the original limits of the muscle. The nuclei in such cases pass through the same chromatolysis as in the above instances.

The late stages of muscular degeneration are seen in the body cavity. Fragments, either carried there by phagocytes, or swept there by some other agent, undergo a fatty degeneration which will be described later. Having thus briefly considered the phenomena found in *Culex*, a discussion of the various points and a comparison with the results obtained by others may be made.

It is the primary factor in this muscular degeneration which appears difficult of determination and upon which authors have expressed materially different opinions based upon observations upon the same or different animals. Kowalevsky ('85) notes the active *interference* of phagoctes within five to six hours after the change to the nymphal stage (*Musca vomitoria*), commencing in the first segments of the body where there is no indication of degeneracy in the muscles; nucleus, sarcoplasm, and striations are absolutely normal from a morphological standpoint. The progress is rapid so that by the seventh or eighth hour all the muscles of the first segment are destroyed.

Bruyne ('98) in the study of the same animal, agrees with Kowalevsky as to the time of the appearance of leucocytes. In nymphs of one and two days, entire muscles were reduced to a conglomerate mass of angular fragments of variable dimensions but having preserved their striations. Almost all are enclosed in leucocytes, while many of the latter, still empty, are found between the fragments of muscular debris. He defines these leucocytes as little protoblasts moving in an amæboid manner, pushing out their pseudopodial extremities through the sarcolemma and into the muscular substance. However, Bruyne does not consider this the beginning of the degeneration, but describes areas showing at once a transition from the muscle fasciculi, still striated, with nuclei in different stages of hypertrophy, to fragmentation of the contractile substance and change in the staining properties of the sarcoplasm, all of which occur without

the presence of leucocytes. He brings this forward to show that the beginning of degeneration is marked by morphological and chemical changes and not by leucocytes. This view which is also upheld by other observers denies that the initial cause of muscular degeneration is phagocytosis, but that phagocytosis is secondary to some other change in the muscle itself.

In *Culex* the appearance of phagocytes about a muscle is a comparatively late occurrence. In the majority of cases there is a considerable loss of substance in the contractile tissue, a vacuolation, without their presence and often they are not found at all. What Bruyne notes in *Musca* and represents in Fig. 1, Pl. VII., of his work, is repeatedly found in this insect, *i. e.*, the muscles show an early degeneration by separation of the fibers and hypertrophy of the nuclei as already shown. The first change noted is a stripping off of the ensheathing layer which often becomes broken, and an increase in the amount of the granular protoplasm which surrounds the nucleus, separates it from the fibers, which, together with a more granular appearance of the sarcoplasm, presents a condition comparable to what is known to pathologists as " cloudy swelling " or " granular degeneration." The nucleus leaves its normal position close to the fiber, where it presents a flattened or oval shape, to lie free in this granular material, and, at the same time, it becomes more spherical in shape. The next step is the loss of striations which gradually takes place, and a division of the fiber into fibrillæ before the form of the fiber is lost. In some instances there is a transverse breaking of the muscle bundle and a localized condensation of the contractile substance as shown in Fig. 2, Pl. X. This is probably due to a contraction of the weakened muscle and is not a constant feature of the process. In Fig. 1, Pl. X., no phagocytes are found. Several nuclei, apparently normal, except for their shape and position, are seen separated from the bundle, and n^1 shows a beginning hypertrophy.

It is evident that in *Culex* there is a direct degeneration of the muscles. Although there is in some cases, not in every instance, a phagocytosis, as will be shown later, it shows no active relation to the degenerative process either in point of time of its occurrence or in the manner in which it manifests itself. Korotneff

('92), Bataillon ('90), Needham ('00) and Breed ('03) all find that phagocytes play no part in the degeneration proper of muscles. Phagocytosis may, or may not occur, but whenever it does it is always a secondary process. No one of these authors attempts to explain the primary cause of the degeneration, though it is variously described as a morphological, physiological and chemical change. Such a change may be accounted for in the subcatobolic conditions involved in a deficient nutritive supply. At the end of physiological activity of the larval organs of an holometabolic insect the nutritive supply is diverted from these organs to the parts which are to evolve the new organs of the imago. Such a process would result in the phenomena above described, a condition which is amply illustrated in certain pathological changes in the tissues of higher organisms when the nutrition is interfered with.

The further phenomena attending the dissolution of the muscles is not constant. As above stated, there appears, in the majority of cases, a dissolution of the muscle substance accompanied by a hypertrophy of the nuclei. In such cases the striation is early lost, the nuclei take an oval or spherical appearance and stain less regularly. In fact the most marked evidence of early degeneration among the larval muscles of *Culex* is the hypertrophy of the nuclei and changes in their morphology and staining properties. Instead of showing a chromatin network and an evenly stained nuclear substance, there are larger or smaller deep-staining granules which soon become arranged about the periphery and contained within a nuclear membrane. The nucleolus disappears and the whole nucleus breaks up by rupture of the membrane and the granules become scattered. Plate X., Figs. 2, 3 and 4, show the evolution of this process. The complete destruction by rupture and dissociation of the granules has been repeatedly seen. Such are the small dark staining bodies found scattered among the other detritus in the body cavity late in the process of degeneration. Their fate is then similar to that of other fragments of muscle tissue to be described later.

In the present study no other condition of the nuclei was observed than that of total disintegration by rupture of the membrane after the entire nuclear substance had become condensed into a few irregular granules at the periphery.

Along with this change in the nuclei there is a rapid destruction of the contractile-substance as above described. In one case by a gradual dissolution, and in the other by the advent of phagocytic cells. In the former instance there is a loss of substance without visible cause, and similar to the phenomena of lyocytosis by which Anglas ('00) accounts for the destruction of larval muscles. It would seem that some unseen agent was producing a digestive action upon the muscles in question. This is illustrated by the conditions represented in Plate X., Fig. 2. This process is marked all through the period of muscular degeneration in all parts of the body, and is undoubtedly the chief factor in the destruction of these organs. What cells are active in secreting an enzyme for the digestion of these inert organs, and why it manifests a selective action upon such tissues is difficult of explanation.

It has been shown that the degeneration of the muscles may be seen making rapid progress without the aid or intervention of phagocytes as contended by authors already cited. These wandering cells, however, make their appearance, and, at times, in considerable numbers, but in proportion to the amount of degeneration taking place their number is indeed small and inadequate to the work to be performed. In fact, it may be said that phagocytes play no part in the degeneration of the muscles in a true sense. After the muscle has become altered in its morphological, physiological, and chemical characteristics, as shown by its loss of striation and separation of nuclei, by its inertness, and by its changed reaction to dyes, and even broken to fragments either by inherent properties of the tissues themselves or by some chemical agent within the body, the debris becomes a foreign body. Wandering cells of the body which have a phagocytic action, whether blood cells or specialized cells derived from mesoblastic tissue, will set about the removal of such foreign material, and in so doing will only be performing their usual physiological function.

The origin of phagocytic cells in *Culex* is not to be attributed to the blood or to the free formation of cells in the degenerating tissues, but to the multiplication of small cells of the connective tissue which are not of a highly specialized character. Plate X.,

Fig. 4, and Plate XI., Fig. 1, besides showing the conditions above described, present several small cells in the granular material about the muscle lying free with the nuclei. They are probably the first appearance of phagocytes, and in this instance, it will be noted, they have entered before the fibers have lost their striations. If one examines the former figure the origin of these cells becomes plainly evident. In the sheath surrounding the bundle are several small elongated cells. Some of these appear large and more spherical, and show by their position and morphological characteristics, their relation to the spherical ones within the sheath though cell division has not been observed. It seems very probable that the phagocytes of *Culex* are derived, not from the blood or myoclasts of Bruyne, but from special mesodermic cells which arise by a proliferation of the cells existing in the body tissues. These cells are later found much larger because of the detritus which they have incorporated, and pass into the body cavity where we shall examine them more carefully a little later.

Of the authors who do not attribute to phagocytosis the initial cause of muscular degeneration, Korotneff ('92) and Bataillon ('90) describe phagocytes as playing a secondary part in the destruction of the debris as described in *Culex*. Breed ('03) says that there may be such a condition but does not note it in his work, while Needham ('00) claims the total absence of phagocytes until the imaginal stage. Breed says the degeneration of the larval muscles is entirely chemical, there being no evidence of phagocytosis. The tracheal cells which he describes, and their ultimate function is quite comparable to the phagocytes above described in *Culex*. In the one the cells are derived from preëxisting cells in the tracheoles and in the other from preëxisting cells in the connective tissue. Both the tracheal cells and the connective tissue cells are of mesodermic origin not specialized to particular functions, and just such cells as one would expect would give rise to phagocytic cells. Again one may find analogous cases in pathology. In the study of inflammatory changes in various tissues a proliferation and phagocytosis is very often observed. The cells which become phagocytic are derived in most instances from either connective-tissue cells or endothelial cells.

Only rarely do the proliferated epithelial cells become phagocytic and then they are of a low, undifferentiated type; they are the flat cells which resemble the endothelial cells and probably perform much the same function. The epithelial cells lining the alveoli of the lungs and of Bowman's capsule of the glomeruli of the kidney may proliferate in certain inflammatory processes and the resulting cells take up a phagocytic function.[1] This would seem to suggest the probable origin of phagocytes and their relation to the muscular destruction in *Culex*. They arise from a proliferation of mesodermic cells, appear after the degeneration has become established and have no causative relation to the degeneration.

Another point which should be mentioned, although not found in *Culex*, is that of the myoclasts described by some observers. Metschnikoff ('83) describes muscular phagocytes in the resorption of the tail of tadpoles, as noted in a preceding paragraph. In *Musca*, Bruyne ('98) also finds instances of the same phenomenon and terms these cells myoclasts. In these instances there are the same initial phenomena as described in the beginning degeneration. The nuclei, however, do not disappear after hypertrophy, but persist and become surrounded by sarcoplasm. This then becomes an amœboid cell and acts as a phagocyte. These myoclasts become very large when distended with numerous sarcolytes. In such a case we have cells arising from the degenerating tissue which attacks tissues of similar origin. The muscles having come to an end of their usefulness, produce the agents of their own destruction. In the study of *Culex* the behavior of the nuclei as above described was constant, *i. e.*, both nuclei and sarcolytes are eventually consumed together in the body cavity. Nothing that corresponds to the description of myoclasts has been observed.

Late in the process of metamorphosis, seen especially in advanced pupæ, but to some extent in earlier forms are masses of sarcolytes or muscle fragments, some free, others enclosed in phagocytes, which show various degrees of degeneration. A very few retain their normal staining properties when treated

[1] "Proliferation and Phagocytosis," F. B. Mallory. From *The Jour. Exper. Med.*, Vol. V., No. 1, 1900, p. 7.

with hæmatoxylin and eosin. From the blue, they pass through a violet to a rose color which markes the necrosis of tissue. Some then give a copper-colored appearance, and finally clear spaces indicate the completion of fatty degeneration. The inference here is that the muscle fragments ingested by the wandering cells are carried into the body cavity where they line the body wall, and complete their work. All through the body beneath the hypodermis, and especially between the muscles of the thorax, large numbers of these cells aggregate, where they are seen scattered among free sarcolytes. Figs. 3 and 4 of Plate X. show the condition just described, Fig. 4 being farther advanced. In some instances a nucleus is found centrally located, while at other times it is pushed to the periphery and flattened to the membrane of the cell by the enclosed fragments. Degeneration seems to take place more rapidly about the nucleus and works gradually to the more distant inclosures.

Two forms of degeneration have been noted, both of a digestive nature, the one extracellular by unseen agents, as if by means of fluids secreted by cells at a distance, or in the muscle itself or some part of it; the second an intracellular digestion which occurs in the instance of ingestion of muscular debris by phagocytes. In either case the result is the same, and probably in both the products are further used in the animal economy in the rebuilding of imaginal tissues. Sarcoplasm and nucleus are both involved in this digestion, though the latter seems more resistant to this agent of destruction and dark staining granules of nuclear material are found late scattered through the body. In connection with this it should be stated that other investigators agree that not all sarcolytes or muscular debris are incorporated in phagocytes. Bruyne ('98) says that many sarcolytes break up without being acted upon by phagocytes. He quotes Loose as giving statistical estimation that there are from 90 per cent. to 96 per cent. free sarcolytes; 4 per cent. to 6 per cent. surrounded by a plasmic envelope, and that 3 per cent. may be found in a plasmic area bearing a nucleus.

In brief, in the process by which the larval muscles degenerate and are destroyed, it has been found that the degeneration proper is a chemical one unaided by any physical action by cells outside

the muscle itself, though it is possible that the muscle is influenced by internal secretions of other cells in the body which reach them through a circulating medium. There is no leucocytic phagocytosis. Phagocytes which apparently arise from the mesodermic tissues, appear in some instances and remove the debris of muscles already broken down, and complete in part, the work already begun by other agents. There is nothing to indicate a myoclastic phagocytosis, or that the muscle gives rise to any organized elements which destroy the remaining tissue by an autophagocytosis, or which go to build up new tissues. The end of the muscle substance is that of conversion into fat or other nutritive material by a process of digestion or chemical change.

Muscular Regeneration.

The manner in which the imaginal muscles arise to take the place of those destroyed has not received as much consideration from investigators as the subject of degeneration. However, as regards those who have studied that part of the process of metamorphosis which has to deal with the development of organs of the imago, a diversity of opinions exists nearly equal to that found in the writings of those interested in the degenerative process. Bruyne ('98) notes the regeneration in *Bombyx mori*. He quotes Viallanes, and reports the same phenomena, *viz.*, a multiplication of muscular nuclei with considerable rapidity, in such a way as to determine areas of little nuclei, sometimes irregularly grouped, sometimes arranged in linear series. He notes mitotic figures here and there, but believes that both direct and indirect division intervene together. A small amount of the sarcoplasm of the larval muscles persists with these nuclei and increases. The subsequent separation of the young nuclei and the striation of the protoplasm contributes to the renovation of the muscular tissue. These cells he terms "myoblasts." Thus in the larval muscles he finds, not only the agents of destruction which he describes as "myoclasts," but also the starting point of the new muscles in the "myoblasts"; so that these two classes of cells, both products of the larval tissues, play a role, physiologically speaking analogous to the osteoclasts and osteoblasts of the higher animals.

Breed ('03) accounts for the wing and leg muscles of the imago by a direct metamorphosis. Carrying the degeneration to a few cylindrical strands of undifferentiated sarcoplasm which contain many nuclei undergoing rapid amitotic division, there is a period of little change followed by the appearance of the fibrillæ of the adult muscle. A little later the elongated nuclei which have formed by the direct division disappear and short oval nuclei are found scattered through the muscle substance. As for the histogenesis of imaginal muscles which are not present in the larva, he gives no definite information. They are probably derived from cells which resemble tracheal cells but have a different origin.

The conditions found in *Culex*, as regards these points, seem rather to confirm the views set forth by Korschelt and Heider ('99). The destruction of the larval muscles is complete. New muscles form from embryonic rudiments present in the imaginal discs. A thickening of the cells at points along the under surface of the hypodermis is found in the thorax, from which, after the degenerative process is well advanced, cells proliferate and extend into the thoracic cavity. These cells lie in irregular masses, but soon form linear series as they extend inward. Mitotic figures have been frequently noted among them. The protoplasm of these cells is not distinct, but large oval nuclei arranged in the line of their long axis appear soon to lie in a syncytium which assumes the shape of a muscle fiber. This condition is represented in Figs. 5 and 6 of Plate XI. Already a longitudinal striation is somewhat apparent in the former, but this becomes more marked in a later stage as shown by the latter figure. Here the amount of protoplasm is increased very considerably and the nuclei are pressed to the side of the fiber, and instead of being the most prominent part of the structure, often show little more than a granular material forming a line along the side of the fiber. The greater number of these nuclei atrophy and disappear at a later period, while the protoplasm continues to increase. An advanced stage which occurs late in pupal life and marks the completion of the morphological characteristics of these muscles by the appearance of transverse striæ is represented in Fig. 4, Plate XI. The nuclei have all disappeared except those which

[illegible]

[illegible] the adult muscle fiber. It [illegible] is though several nuclei of [illegible] the mature nucleus, but it was [illegible] of cases to state that such is

[illegible] the new muscles from imaginal [illegible] the sections studied, but seems [illegible] when one considers the general [illegible] of organic development among [illegible]ism.

[illegible] CONCLUSIONS.

[illegible] muscles begins in the thorax when [illegible] and is due to chemical alterations. [illegible] factor, though a lyocytosis may

[illegible] the muscular detritus after the muscle [illegible] by a chemotaxis, and remove a part

[illegible] not blood cells, but probably of meso- [illegible] a proliferation of cells scattered through

[illegible] muscular destruction is the digestion of [illegible] the phagocytes (intracellular) or by a fatty [illegible] fragments which are not ingested by cells, or [illegible] of a fluid secreted by cells elsewhere in [illegible]

[illegible] autophagocytosis of muscles. No myoclasts [illegible] the other hand, the nuclei undergo a disin- [illegible] along with the contractile substance [illegible]

[illegible] of new muscles of the adult is from the [illegible] a proliferation of the embryonic cells which [illegible] undifferentiated during the larval growth.

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DESCRIPTION OF PLATES.

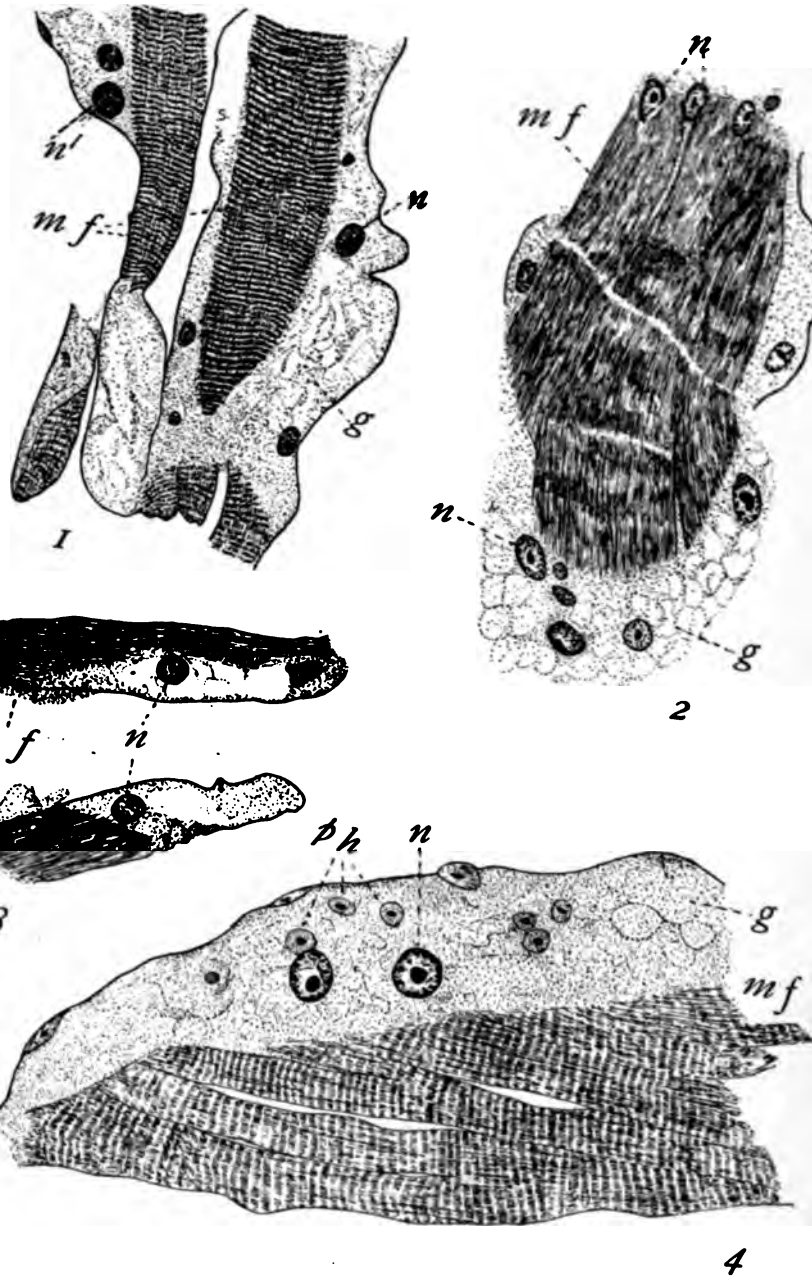
EXPLANATION OF PLATE X.

FIG. 1. *Culex pungens*, larva two thirds grown, showing the beginning of degeneration. Several nuclei (*n*) are seen in the granular material (*g*) separated from the muscle fibers (*mf*). *n'* shows a hypertrophy and nearly spherical form. The smaller nuclear bodies are shown in preceding and following sections to be similar nuclei to those shown in this section.

FIG. 2. The muscle reduced to fribrillæ with marked breaking and condensation of parts. Many hypertrophied nuclei are also seen.

FIG. 3. Here is represented a marked degree of disintegration of the muscle substance and vaculation without the presence of granular material.

FIG. 4. This shows the same as Fig. 1 with many small cells in the connective tissue envelope of the bundle.


n'
mf
n
g
1
n
mf
n
g
2
mf
n
3
ph
n
g
mf
4

EXPLANATION OF PLATE XI.

FIG. 1. Representing a small portion of a muscle surrounded by an increased amount of granular material in which are seen, besides a single enlarged and degenerate nucleus *n*, several small mesodermic phagocytes *ph*.

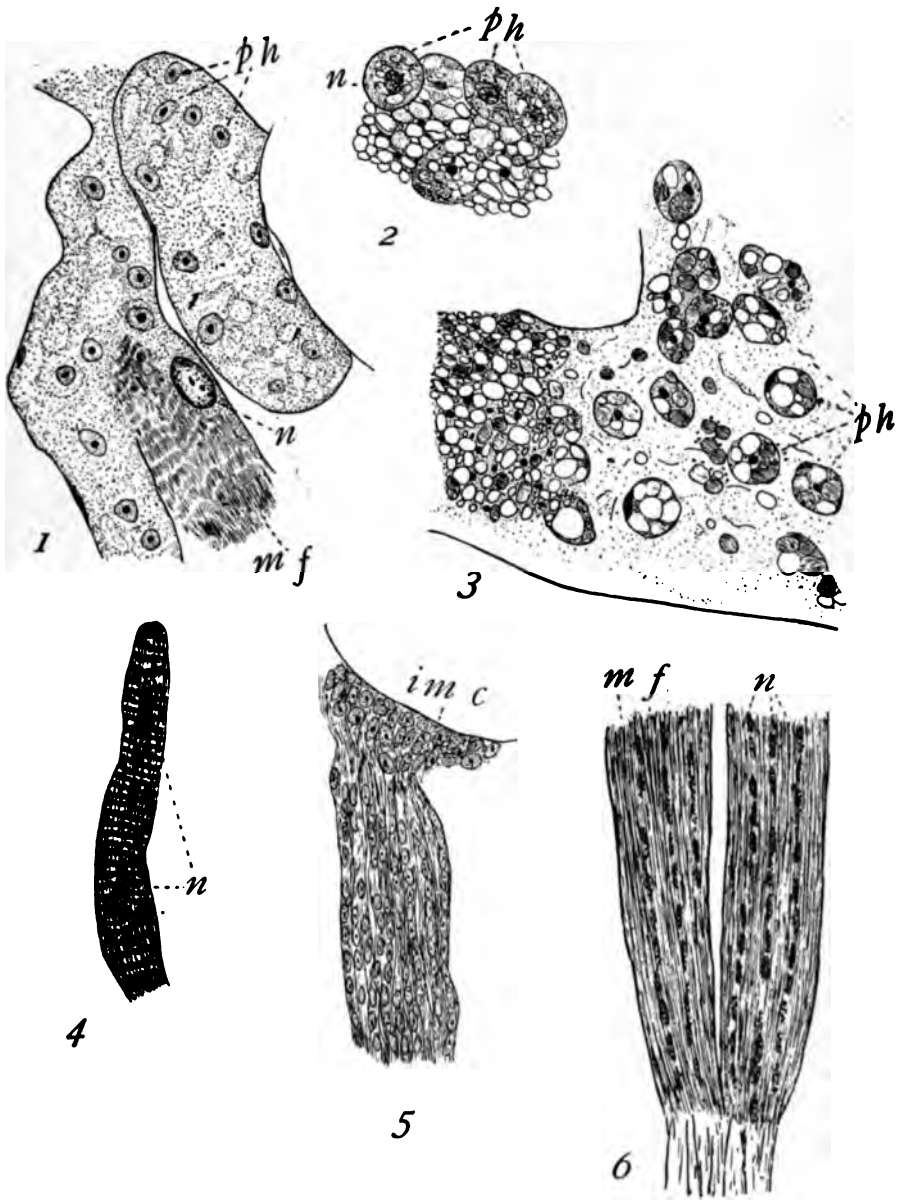
FIG. 2. The section shows a mass of sarcolyted and engorged phagocytes, the former undergoing destructive changes both within and without the cell.

FIG. 3. Similar to Fig. 2, though showing several isolated phagocytes with phagocytes *ph* with nuclei either centrally placed or crowded to the periphery. To the left is seen a formless mass of detritus.

FIG. 4. Showing final stage of the regenerated muscle fiber with striations.

FIG. 5. Showing the beginning of the formation of the new muscles of the adult. A proliferation of cells from the imaginal disc and the arrangement of the protoplasm into the form of fibers.

FIG. 6. This shows a later stage than Fig. 5, with an increase in the amount of protoplasm and the arrangement of the nuclei to the sides.


ph
n
m f
1
ph
n
2
ph
3
n
4
im c
5
m f
n
6

ECOLOGICAL NOTES ON THE MUSSELS OF WINONA, PIKE, AND CENTER LAKES OF KOSCIUSKO COUNTY, INDIANA.[1]

THOMAS J. HEADLEE.

The Mussels of Winona Lake.

During the summer of 1902 I became convinced that the mussel fauna of Winona lake had a definite distribution, which would repay careful study. I proposed, should circumstances permit, to study this distribution and the conditions which control it. The opportunity came the following summer and with the aid of Mr. James Simonton, who had become interested in the problem, the study was undertaken.

We examined the bottom from four inches to four feet by wading, from four to seven feet with a clam rake, from seven to eighty-six feet with an iron dredge.

The species found were determined by comparison with shells that had been named by Call, Simpson, and Baker. The nomenclature is that used by Call in in his "Catalogue of the Mollusca of Indiana," which was published in the Indiana Geological Report for 1899. They were: *Unio luteolus* Lamarck, *Unio subrostratus* Say, *Unio glans* Lea, *Unio fabalis* Lea, *Unio rubiginosus* Lea, *Anodonta grandis* Say, *Anodonta edentula* Say, and *Margaritana marginata* Say.

Winona is a deep kettle-hole lake. In general, the beaches are composed of sand and gravel, which shade off with varying rapidity into marly sand, then into sandy marl, then into coarse white marl, and finally into the soft dark mud that covers the bottom in all the deeper parts of the lake. The bottom steadily grows softer as the proportion of dark mud increases. So soft does it become that a small sounding-lead sinks into it of its own weight from six to twelve inches. However, in some places, especially the southwest side of the large lake and in nearly all

[1] Contribution from the Zoölogical Laboratory of Indiana University, No. 75.

parts of the small one, the shallow part of the beach is formed of muck which shades off into marl without the presence of any sand or gravel.

In general, it may be said that the mussel zone extends from the shore-line to where the bottom changes to very soft mud. This region is covered by from four inches to nine feet of water, although in some places the mud comes to within a few feet of the water's edge, while in others the sandy and gravelly bottom runs out into twenty-two feet of water.

A. grandis is found just on the outer edge of the sandy and gravelly banks, while *A. edentula* appears most abundantly a little farther out. A few specimens of both species were taken closer inshore, *grandis* being sometimes found on sandy bottom, *edentula*, however, invariably upon soft bottom. Neither (healthy forms) was taken on hard sand or gravel. *U. glans* has been taken upon sandy and gravelly bottom in from four feet out. *U. fabalis* appears in about the same region, except that it goes out on the soft bottom as far as edentula. *U. subrostratus* appears on the outer edge of the sandy and gravelly banks in about four feet of water, and also further inshore where the bottom is soft. *U. luteolus* is the most variable, the most widely distributed, and the most abundant of all the species in the lake. It varies from a moderately thin, light straw-colored shell, marked by radiating greenish lines, to an extremely heavy, almost black form. The gradations of form, color, and size are shown in Figs. 9–19 in the plate. The straw-colored variety is found in from four inches to twenty-two feet of water. It is, however, dominant inshore, in weed patches (*Potamogeton* and *Ceratophyllum*), and on chara-covered bottoms. The dark variety occupies the same region but is dominant upon sandy and gravelly bottom in from three and one half to twenty-two feet of water. The intergrading forms cover the same territory as the straw-colored and dark varieties but cannot be said to be dominant anywhere. *U. rubiginosus* occupies about the same habitat dominated by the dark form of *U. luteolus*, except that it was not found in water deeper than ten feet. *M. marginata* was found so infrequently (only six times) that we could tell little of its distribution. The

specimens found were taken on sand and gravel and white marl bottoms in from four to twenty-two feet.

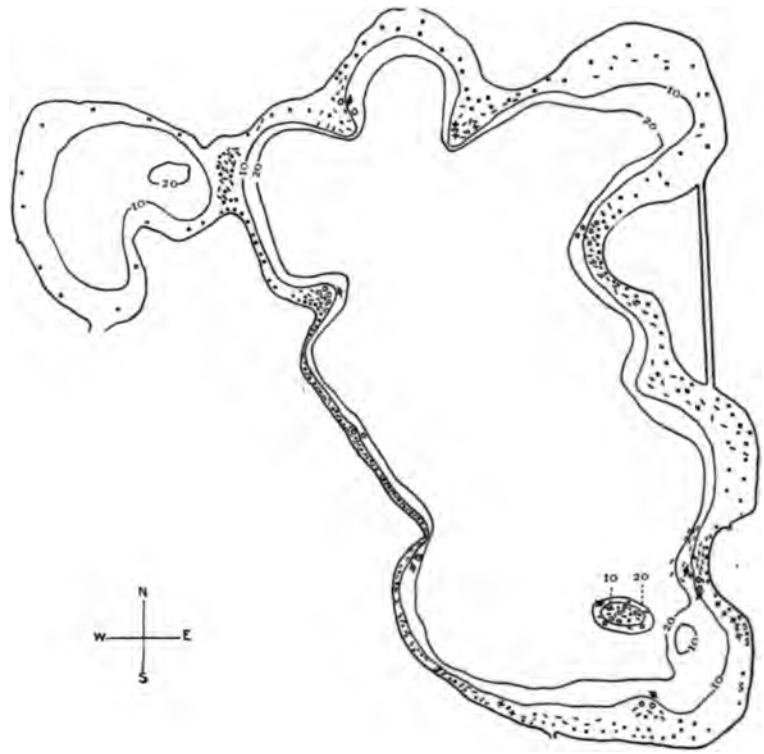


FIG. 1. Map of Winona Lake showing mussel beds. ● = *U. luteolus* — light variety; — = *U. luteolus* — dark variety; ○ = *A. grandis*; ϕ = *A. edentula*; ÷ = *U. rubiginosus*; S = *U. subrostratus*.

There are a number of conditions which suggested themselves as possible explanations for this distribution — sex, light, temperature, food supply and oxygen, pressure, wave action, character of the bottom, and enemies. Sex cannot be important, for males and females are found together throughout the habitat; light can have but little to do with it, for mussels are absent in places in three feet of water and are abundant in others in fifteen feet, the difference in light being considerable. Further, the light over some of the immense beds in White River is no greater and perhaps even less than in twelve feet of lake water. That heat and cold have little effect, during the summer at least, is shown by

the fact that heavy beds were found in different temperatures, and by the fact that temperature variation in the mussel zone did not amount to more than two degrees; oxygen is not important, for the supply of oxygen throughout the mussel zone varies very little; pressure can have but little to do with it, for we found specimens on a sandy bottom in twenty-two feet of water, while on dark mud bottoms in ten feet none were taken in any case. Food supply cannot be effective, for it is about equally abundant throughout the zone. The food consists of diatoms and other low algæ forms and one-celled animals.

It seems to us that there are three factors which control the distribution of the mussels in Winona lake — wave action, character of the bottom, and enemies.

The first factor is active only in water less than three feet deep. As *U. luteolus* and *A. grandis* appear in this region they are subjected to this agency. Specimens of both *A. grandis* and the dark form of *U. luteolus* have been found washed ashore after a storm, and scores of these shells appear along the shore-line. Under similar conditions we have seen the light form of *U. luteolus* moving from the water's edge out into deeper parts. These facts point to the conclusion that the two first mentioned forms are, in general, prevented from occupying shallow water by wave action, but that the light form of *U. luteolus*, being very active and having a relatively thick shell, can well occupy this region. *U. glans*, *U. fabalis*, *U. subrostratus*, *U. rubiginosus*, *A. grandis* and *A. edentula*, if washed ashore would be unable to get back, and the shells of the last two would quickly be broken through by wave action. Those forms which occur in this region, especially those to which washing ashore is fatal, habitually bury themselves so deeply in the mud that only the siphonal tip of the shell projects and thus they are protected from the dragging action of the waves.

The character of the bottom applies throughout the mussel zone and is by far the most important factor. The bottom in the weed patches differs from that in the deeper parts of the lake in being less soft. The sandy and gravelly bottom affords firm foothold and allows the mussel to assume that position which enables it to get the best supply of food and oxygen, while the pure black mud allows it to sink so far as to be smothered.

In order to test the ability of the mussel to withstand these bottom conditions we made three wire clam baskets. One we lowered in twenty-five feet of water, another in thirty-five feet, and the other in eighty-five feet. The basket in twenty-five feet was placed on August fifth on a dark mud bottom. It contained thirteen *U. luteolus* and one *A. grandis*. On the tenth two of *U. luteolus*, dark variety, were dead; on the fifteenth one of *U. luteolus*, dark variety, was dead; on the seventeenth two of *U. luteolus*, dark variety, were dead and four were missing. The basket in thirty-five feet was placed on a sandy gray marl bottom on August ninth. It contained five *U. luteolus* of the light variety and one of the dark, and one *A. edentula*. On the fifteenth one *A. grandis* and one *U. rubiginosus* were added. On the twentieth one *U. luteolus* of the dark variety was dead; on the twenty-fourth five *U. luteolus* and one *U. rubiginosus* were found to have the gills badly choked with sediment, while the Anodontas were missing. The eighty-five feet basket was lowered on pure dark mud on the fifteenth of August. It contained seven *U. luteolus* of light and one of dark variety, two *A. edentula* and one *A. grandis*. On the twenty-first one *U. luteolus* of dark variety was dead; on the twenty-fourth seven *U. luteolus* and one *A. graudis* showed gills badly choked with sediment, while the two *A. edentula* were in better condition, showing very few patches of mud in their gills.

This experiment, incomplete as it is, serves to show two important facts—that the dark variety of *U. luteolus* resists the conditions of the gray marl far better than those of the fine black mud bottom, regardless of depth of water, for in the twenty-five feet basket on dark mud in five days two specimens, and in the eighty-five feet basket on dark mud in six days one specimen, were killed, while eleven days were required in the thirty-five feet basket on gray marl to destroy a single specimen; that the dark variety of *U. luteolus* is least resistant to these bottom conditions. It also indicates that the *Anodontas*, especially *A. edentula*, resist better than any other kind experimented with.

Naturally one should expect to find some difference in structure by which the mud is more thoroughly excluded in the more resistant species than in those which suffer more severely. There

is at least one such difference — the size of the shell in proportion to its weight. The Anodonta shells are very light, while those of the dark form of *U. luteolus* are very heavy in proportion to surface exposed. Many shells of the former, especially those of *A. edentula*, are easily crushed between the thumb and fingers, while those of the latter will frequently withstand the heavy blow of a hammer. The former is evidently fitted for a life on soft bottom, while the latter finds a most congenial situation on coarse sand or gravel beds. Between these two extremes the other species range, and indeed we find the main facts of their distribution explained by the status of this condition.

Accordingly *A. grandis* and *A. edentula* are found on the outer edge of the sandy marl banks, the *edentula* being better fitted to withstand the bottom conditions, even out in the edge of the dark mud. *U. glans* and *U. fabalis*, owing to lightness, are able to occupy about the same region. They are also found inshore in situations not subjected to wave action. *U. subrostratus*, having medium weight valves, appears on gravel and sand banks, in weed patches, and on chara-covered beds. *U. rubiginosus*, having very heavy valves, is confined to clear sand and gravel banks. The straw-colored form of *U. luteolus*, on account of its medium weight valves, is able to live on sand, gravel, in weed patches, and on chara-covered beds. Owing to the fact that so few specimens of *M. marginata* were found, we were unable to draw any conclusions as to its ecology.

The muskrat is the principal enemy of the mussel; around his house many mussel shells are found but no live mussels. Shells of all the species in the lake except the smaller ones appear there, those of the *Anodontas* being in much greater evidence than is proportionate to their total number. They do not appear so on first examination, for they are broken up by the animal and worn by the waves. The conditions on the sand banks beyond reach of wave action are very favorable for *Anodonta* life, except for the presence of the muskrat. They are absolutely absent from the water some distance from his home where we found Unios rather abundant. This points to the fact that the muskrat confines the *Anodonta* to the deeper water at the edge of sandy and gravelly banks.

It seems to us that the foregoing facts give basis for the following conclusions: the mussel zone lies mainly upon sandy and gravelly banks, and on the outer edge of the same; wave action and the muskrat determine the shoreward limit of the distribution, and the character of the bottom is the principal factor determining the outer boundary of the zone.

The Mussels of Pike and Center Lakes.[1]

My work in the summer of 1904 was carried on with the aid of Mrs. Headlee and was simply a continuation of that of the previous summer. We had two main objects in view in pursuing this study further; to determine which of the distributional forces discovered in Winona lake may be essential, that is, may apply to small fresh-water lakes in general; to ascertain by experiment the effect of black mud bottom conditions on mussel life, and what species are most resistant.

In pursuance of the first object we studied the mussel distribution in Pike and Center lakes and I shall devote the following paragraphs to a brief discussion of them.

In Center Lake we have Winona in miniature. Some new elements, however, enter in the form of sewage-like shore washings on the southeast side and the absence of *Potamogeton* patches and of heavy wave action.

In general, I may say that the mussels here are much fewer as compared with the number in Winona than the difference in the sizes of the lakes would warrant. There are two conditions here which may safely be regarded as at least partial explanations of this difference: the southeast shore, which once supported great numbers, has become unfavorable to mussel life through the accummulation of decaying vegetable and fæcal matter; there is an enormous destruction by the small boy, who finds amusement in collecting mussels to throw or to cut open for pearls.

Such mussels as are found, all varieties of *U. luteolus*, *U. subrostratus*, *U. rubiginosus* and *A. grandis*, appear in situations in

[1] Pike and Center are two small lakes within two miles of Lake Winona and at the very edge of the town of Warsaw, Indiana.

almost perfect agreement with those of Winona. There are, however, two noteworthy differences — very few mussels occur in water deeper than twelve feet, although our dredge scraped over gravel in seventeen feet; very few are to be found in water less than three feet deep. I have no explanation to offer for the first variation; the second is undoubtedly due to human agency.

The mussel zone in Center Lake, therefore, extends over gravel, sand, and white marl banks in from three to twelve feet of water. The deep-water edge is determined by the character of the bottom, modified locally, doubtless, by some as yet unknown factor, while the shoreward edge is determined by the ravages of small boys. Neither the muskrat nor the action of the waves is effective here for the first is not present and the second is not powerful enough. That the shoreward limitation is due to man is shown by the fact that only those individuals of the deep-burrowing species which best exemplify this tendency, are able to survive in the area from three feet to the water's edge. So rigid is this selection that we looked long and carefully before we found any mussels at all in this region. We found the light colored form of *U. luteolus*, *U. rubiginosus*, and *U. subrostratus*, and in every case the mussel was buried so deeply that only the extreme tip of the shell projected. Even this was frequently covered with a spongy growth, which still more effectually concealed it.

Pike Lake, in size, falls between Center and Winona and in depth it does not exceed the former. The bottom conditions resemble those of Winona and considerable diversity of beach obtains. All the species found there, except the Corbiculidæ, which we did not seek, were taken here. Everything seemed favorable for a large bivalve fauna, yet mussels are almost as scarce as in Center. I can give no complete explanation for this condition, although here, too, human agency is certainly a very important factor.

The mussel zone extends from a depth of four feet to fifteen feet on the sandy and gravelly beaches; on gray marl beaches its shoreward edge extends into shallower water. The deep water edge is determined by the character of the bottom, the

shoreward edge by wave action, the muskrat, and human agency. Wave action would keep the clumsy and slow-moving forms out of the region from eighteen inches to the water's edge; the muskrat would keep mussels scarce about his house, as indeed he has; but neither nor both could keep them absent from favorable beds in from four feet to eighteen inches of water. This peculiarity of distribution is noticeable only on sandy and gravelly beaches, such as are frequented by bathers. We have seen these throwing mussels on shore and out into deep water and therefore believe this to be the cause of the dearth of mussels in these regions.

In Winona, Pike and Center lakes, mussels are most numerous on the sandy and gravelly beaches; they are scattered on gray marl; very rare on muck; and are not present at all on the fine black mud. They thrive best on gravelly and sandy beds, poorly on gray marl, and not at all on muck and fine black mud. The primary and essential factor in mussel distribution in these three lakes is the character of the bottom, while wave action, the muskrat, and human agency play varying and secondary parts.

Experiments.

The work done in 1903 seemed to show that bottom conditions are favorable or unfavorable as they do or do not allow the animal to assume his natural position and to project his siphons above the mud. When he cannot get his siphons above the mud, his gills become choked and death follows. The animal, therefore, which remains near enough the top of the mud to project his siphons is bound to be the most successful in resisting it. Any of the mussels can do this on sandy or gravelly bottoms, but where the bottom is composed largely of the fine black mud, only such forms as *U. fabalis*, *A. grandis* and *A. edentula* are able to sustain themselves. These shells are all light and the last two expose a large surface in proportion to weight.

We placed eight baskets in water from nine and one-half feet on sandy bottom to eighty-two feet on fine dark mud. Of these, two were lost within a week, one in four weeks, and one in eight weeks and three days. Four remained until the contents were taken out, killed, and examined. We further carried on an ex-

periment in a common wash-tub to which I shall refer in detail later.

In these experiments when a mussel was found, whose gills contained mud, we considered that he was the victim of bottom conditions. So far as our experience goes, the presence of mud in the gills is always followed by the death of the animal if he cannot free himself from the environment which is responsible for his mud-choked state. Beginning with the least successful, the following forms are given in the order in which they stood the bottom test, assuming that the more mud they contained, the less well had they withstood the soft bottom: The dark form of *U. luteolus*, *U. rubiginosus*, the medium form of *U. luteolus*, the light form of *U. luteolus*, *U. subrostratus*, *A. grandis* and *A. edentula*. *U. glans* and *U. fabilis* have not been listed because we were unable to obtain a sufficient number for investigation.

Since the mussels taken directly from the beds differed from those taken from the baskets placed on dark mud in that the latter showed from sixty to ninety per cent. of the total number choked with mud, the question might be raised, is not the presence of mud due to the animal being confined in a basket, where, perhaps, he cannot place himself most advantageously? The confining of the mussels in the baskets might operate unfavorably through crowding or through lack of soil in which to assume their normal position. The basket in all cases was made large enough to give each mussel more room than he would have on a crowded bed. It is true that on a hard bottom the basket would prevent the animal from burrowing in the soil, but on the soft bottom he would have from two to six inches of it. The baskets were weighted so that they would be forced down into the soft bottom. We set a basket on a gravelly sand bottom, but it was lost just before we would have killed and examined its contents. However, during the forty-five days of its submersion before its loss, none of the mussels died — a condition which obtained in but one of the baskets planted on mud, and this one was in but twenty feet of water where the bottom was not so soft as in the deeper parts. The mussels in the basket on gravelly sand were subjected to more injurious conditions by the basket itself than were those of the others, for the wire in this case could not sink

into the bottom enough to give them soil in which to right themselves.

We placed three specimens of each species experimented upon, except *U. rubiginosus*, in a tub, which had previously been two-thirds filled with fine dark mud from the deep area, and then filled up with water. This water was freshened by the use of *Elodea* and the occasional addition of clean water, which was so added as not to disturb the mud. After seventeen days all of *U. lutcolus*, two of *U. subrostratus*, and one of *A. grandis* showed mud in gills, thus effectually demonstrating that the basket was not alone to blame for the general failure to withstand the fine black mud of the bottom.

The work of 1903 and 1904 shows conclusively that the mussels of Winona, Pike, and Center lakes cannot exist on the fine black mud bottom — they become choked with mud and apparently smother — and that the light weight forms and the forms exposing great surface in proportion to weight can rest on top of comparatively soft mud and can, therefore, live farthest out on the deep water edge of the bed. Because the mussels cannot occupy any region where the pure black mud is present, they are confined by it to isolated beds and narrow bands of shore-line.

I believe that the whole evidence of the distributional and experimental work of 1903 and 1904 points clearly to the character of the bottom as the great basal influence in the distribution of mussels in small lakes generally.

Additions to the Known Fauna.

We knew that various species of *Sphærium* had been taken in Turkey and Maxinkuckee lakes and therefore hoped to find more than the one thus far reported from Winona.

Mr. A. M. Banta told us of a small bivalve that he had discovered in an artificial lily-pond in the assembly grounds near the lake. It required but little effort to find it in the decaying vegetable matter in the bottom of this pond, but we did not take it in the lake. It is *Sphærium partumeium* Say. It has not been reported from this region before. The dead shells of *Sphærium striatinum* Lamarck occur in great numbers along the

shores of the lake and even in deep water but, in spite of considerable search, we found no live specimens. The shells of *Pisidium inæquilaterale* Prime were also found, although less abundantly, but neither were live specimens of it taken. Neither of these has before been reported from this region and the latter, not from Indiana.

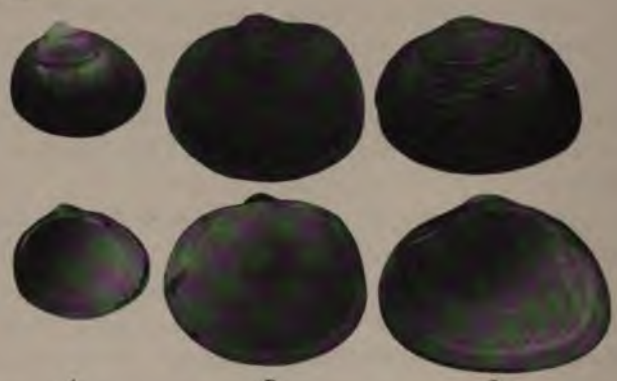


FIG. 2. *A*, left and right valves of *Pisidium inæquilaterale* (dorso-ventral diameter of original is .19 in.); *B*, left and right valves of *Sphærium partumeium* (dorso-ventral diameter is .29 in.); *C*, left and right valves of *Sphærium striatinum* (dorso-ventral diameter of original left valve is .27 in., while that of the original right valve is .29 in.).

In conclusion, I wish to acknowledge my indebtedness to Prof. C. H. Eigenmann under whose direction this work was done, and to R. Ellsworth Call, who kindly identified the three last-mentioned species.

EXPLANATION OF PLATE XII.

FIG. 1. Right and left valves of *Unio fabalis*.

FIG. 2. Right and left valves of *Unio glans*.

FIG. 3. Right and left valves of *Unio subrostratus*.

FIG. 4. Right and left valves of *Unio rubiginosus*.

FIG. 5. Right and left valves of *Margaritana marginata*.

FIG. 6. Right and left valves of *Unio luteolus*.

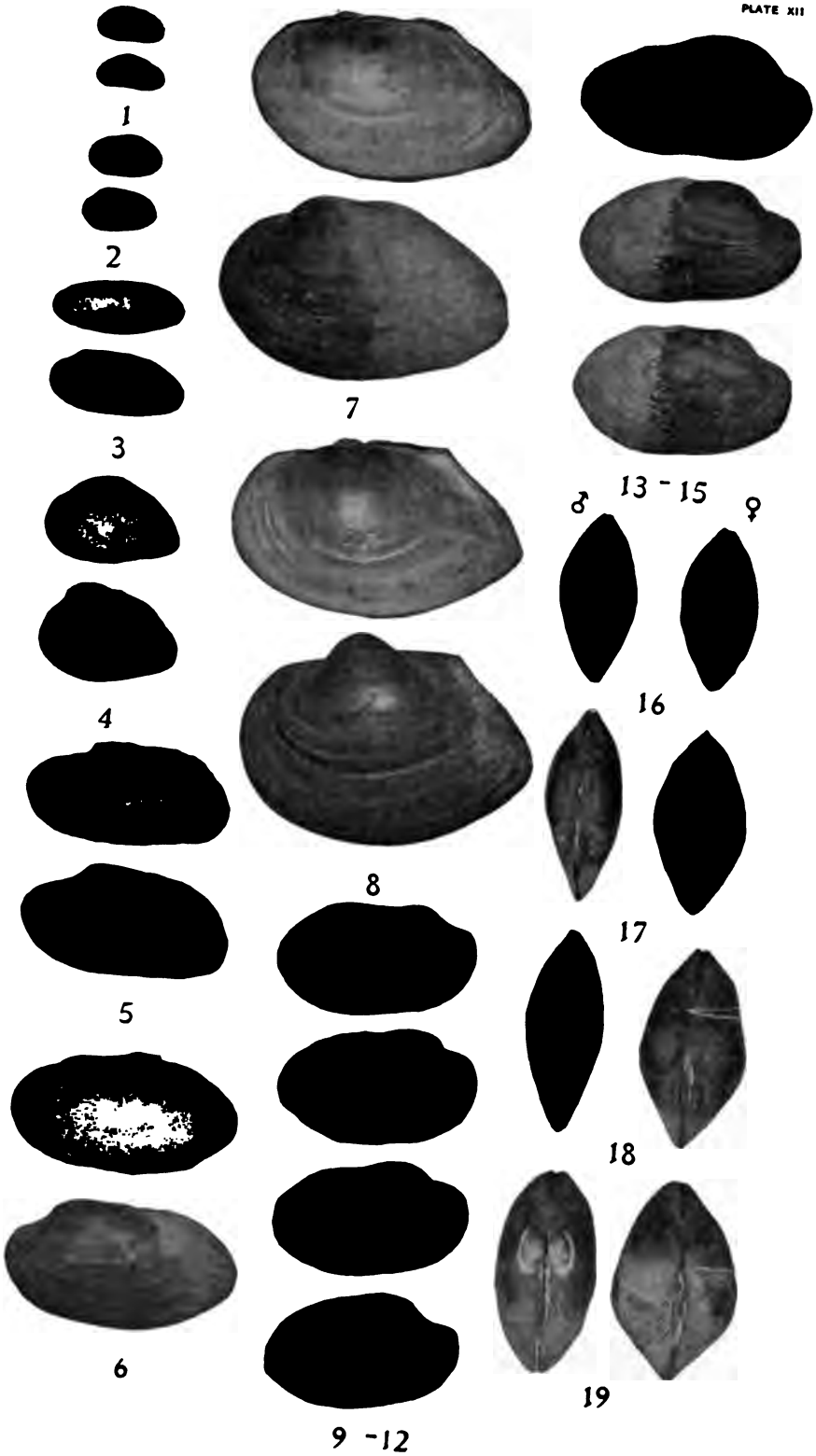
FIG. 7. Right and left valves of *Anodonta grandis*.

FIG. 8. Right and left valves of *Anodonta edentula*.

FIGS. 9–15. Side view of a series showing gradations in form, color, and size from the small light-colored variety to the large dark-colored variety of *Unio luteolus*.

FIGS. 16–19. Dorsal view of a series of *Unio luteolus* including both males and females and showing the gradations in form and size from the small light-colored to the large dark-colored variety.

Note. — The shell represented in FIG. 7 is 2.75 in. from dorsal to ventral surface and 4.5 in. from anterior to posterior end. The reduction of FIGS. 1–19 is the same as that of FIG. 7.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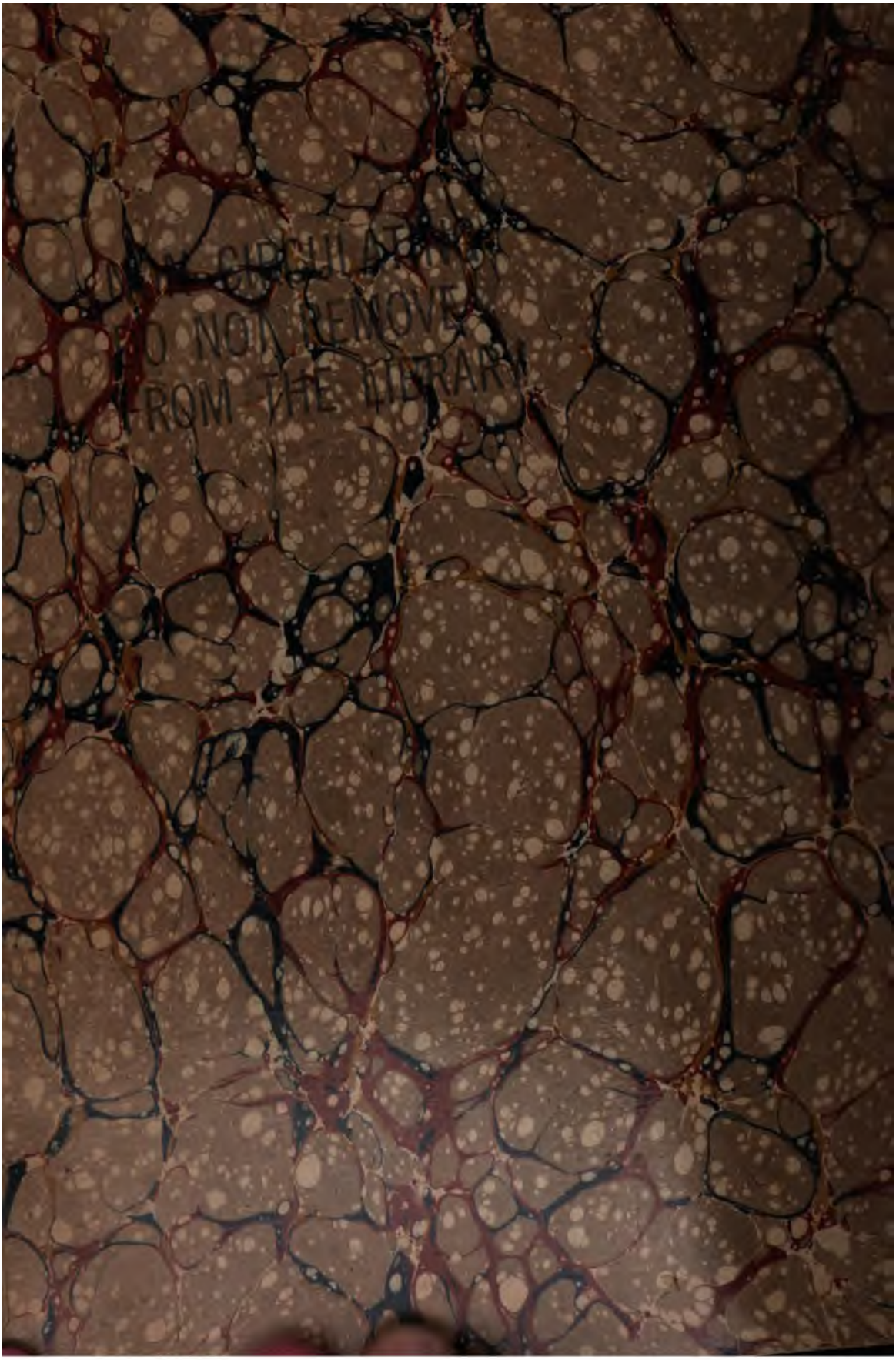
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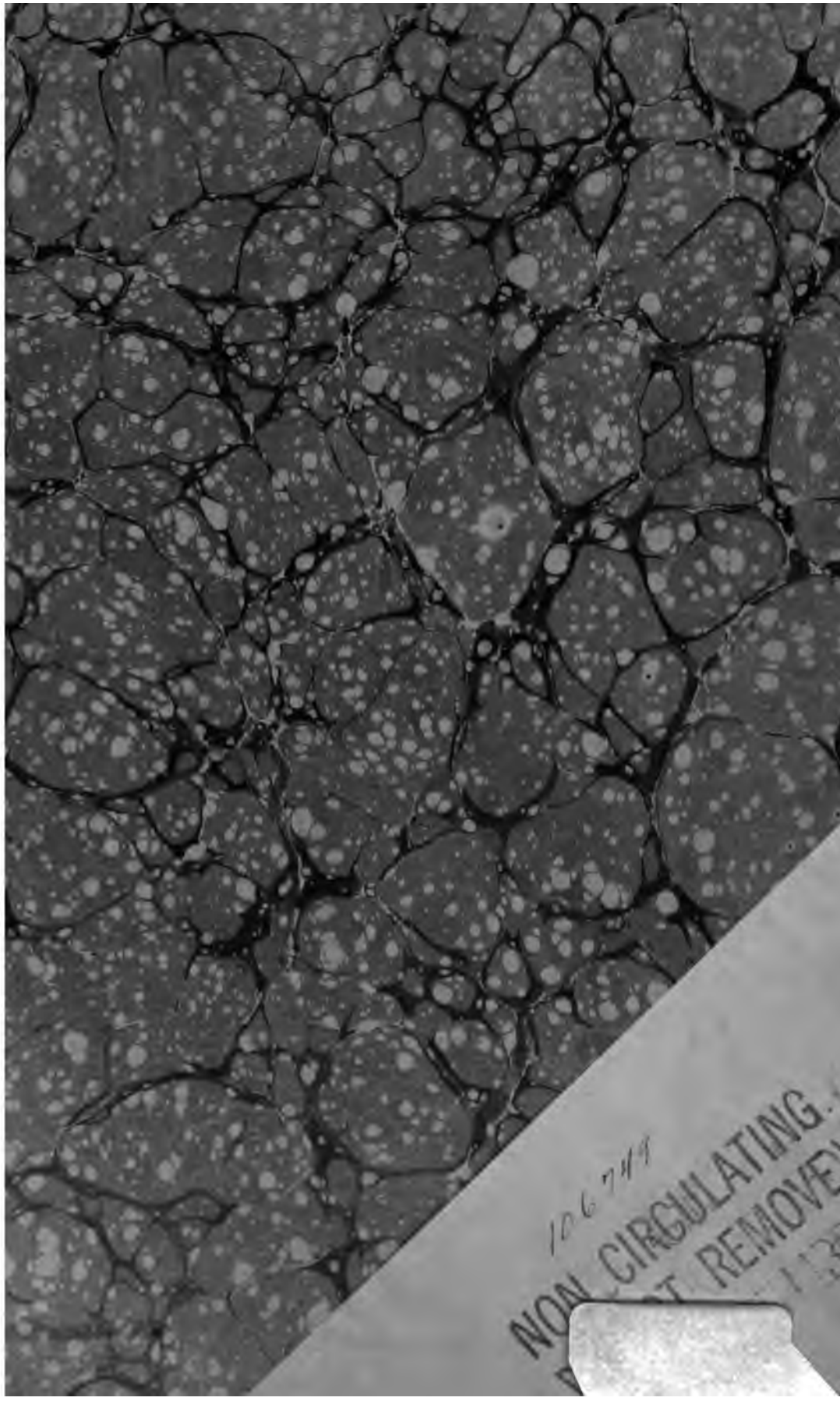
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NON CIRCULATING
REMOVE